INTRODUCTION

Gastric cancer (GC) remains the fifth most frequent cancer and the third leading cause of cancer death worldwide in 2018. Gastrectomy-based perioperative or postoperative adjuvant therapies have been applied, but prognosis remains unsatisfactory. Peritoneal dissemination (PD) is the most common cause of tumor progression in advanced GC, and the median survival of patients is only 4-6 months. However, the mechanisms underlying PD are not entirely clear. Furthermore, there is a lack of accurate diagnostic biomarkers and excellent therapeutic targets for PD.

Gastric cancer peritoneal dissemination (GCPD) is the result of interactions between tumor cells and the peritoneal mesothelial cells (PMCs). The injury of mesothelial cells plays an important role in GCPD. However, its molecular mechanism is not entirely clear. Here, we focused on the sphingosine kinase 1 (SPHK1) in human peritoneal mesothelial cells (HPMCs) which regulates HPMCs autophagy in GCPD progression. Initially, we analyzed SPHK1 expression immunohistochemically in 120 GC peritoneal tissues, and found high SPHK1 expression to be significantly associated with LC3B expression and peritoneal recurrence, leading to poor prognosis. Using a coculture system, we observed that GC cells promoted HPMCs autophagy and this process was inhibited by blocking TGF-β1 secreted from GC cells. Autophagic HPMCs induced adhesion and invasion of GC cells. We also confirmed that knockdown of SPHK1 expression in HPMCs inhibited TGF-β1-induced autophagy. In addition, SPHK1-driven autophagy of HPMCs accelerated GC cells occurrence of GCPD in vitro and in vivo. Moreover, we explored the relationship between autophagy and fibrosis in HPMCs, observing that overexpression of SPHK1 induced HPMCs fibrosis, while the inhibition of autophagy weakened HPMCs fibrosis. Taken together, our results provided new insights for understanding the mechanisms of GCPD and established SPHK1 as a novel target for GCPD.

KEYWORDS
autophagy, gastric cancer peritoneal dissemination, mesothelial cell, SPHK1
peritoneal microenvironment. Our previous studies clarified that transforming growth factor-beta 1 (TGF-β1) from GC cells stimulated human peritoneal mesothelial cell (HPMC) fibrogenesis. SPHK1, as a critical mediator of GCPD, promoted tumor cell adhesion and invasion. Recently, TGF-β1 has also been reported to regulate autophagy, a process of bulk degradation of intracellular components through the formation of autophagosomes and degradation by lysosomes. Autophagy is critical for the homeostasis for normal proliferation and differentiation, and it is also an adaptive response to maintain cellular viability after exposure to stressful stimuli. Interestingly, it has been demonstrated that TGF-β1 differentially regulates autophagy; specifically, the growth factor promotes autophagy in vascular endothelial cells and tubular epithelial kidney cells, but inhibits this process in fibroblasts from patients with idiopathic pulmonary fibrosis. However, the autophagy level of HPMCs, especially in the GCPD microenvironment, remains largely unclear.

Sphingosine kinase 1 (SPHK1) catalyzes the phosphorylation of sphingosine to sphingosine 1-phosphate. SPHK1 is widely involved in cell growth, proliferation, and apoptosis. In particular, SPHK1 plays an oncogenic role in promoting survival and invasion in some tumors. High SPHK1 expression promoted breast cancer cell proliferation and invasion, which were associated with poor overall survival. Additionally, SPHK1 participated in cisplatin and docetaxel resistance in gastroesophageal cancer. However, little is known about the role of SPHK1 in tumor stroma cells. Emerging evidence has implicated SPHK1 in cellular autophagy in pathological conditions. Nevertheless, the precise roles of SPHK1 in HPMCs autophagy, in addition to the regulatory mechanisms and the relationship of SPHK1 with GCPD, should be confirmed.

In the present study, we found that overexpression of SPHK1 in HPMCs was associated with LC3B expression (an autophagy protein marker), peritoneal recurrence, and poor overall survival. Using a coculture system, we observed that GC cells secreted TGF-β1, which promoted HPMCs autophagy by regulating SPHK1. In addition, SPHK1-driven autophagy accelerated the occurrence of GCPD. Moreover, we explored the relationship between autophagy and fibrosis in HPMCs, observing that the regulation of HPMCs fibrosis was partially induced by SPHK1-induced autophagy.

2 | MATERIALS AND METHODS

2.1 | Patient tissue specimens

In total, 120 patients with GC who underwent radical surgery at the First Hospital of China Medical University between 2003 and 2010 were included in this study. Resected peritoneal tissues were fixed and embedded in paraffin. All patients were approved for study participation by the Ethics and Indications Committee of China Medical University. Written informed consent was provided for all patients. The TNM stage of the patients was restaged according to the eighth edition of the AJCC cancer staging manual.

2.2 | Immunohistochemistry

Immunohistochemistry (IHC) staining was performed in line with the routine protocols. Sections were deparaffinized using xylene and hydrated through an ethanol gradient. Endogenous peroxidase activity was blocked by incubation in 3% H2O2 for 15 minutes, and antigen retrieval was conducted using 0.01 mol L−1 sodium citrate buffer (pH 6.0) for 3 minutes at high pressure. After blocking in 10% normal goat serum, sections were incubated with a primary antibody against SPHK1 (1:200, Abcam, Cambridge, MA, USA) LC3B (1:400, Abcam, Cambridge, MA, USA) at 4°C overnight. Sections were incubated with HRP-conjugated secondary goat anti-rabbit antibody at 37°C for 30 minutes and exposed to 3,3′-diaminobenzidine. Subsequently, sections were counterstained with hematoxylin. Staining was judged by the percentage of positive cells and staining intensity. The percentage of positive cells was scored as follows: <5% (0), 5%-25% (1), >25%‐50% (2), >50%‐75% (3), and >75% (4). The scoring system for staining intensity was as follows: negative (0), weak (1), moderate (2), and strong (3). The staining index was calculated by multiplying the score for the percentage of positive cells by that for staining intensity. An index of 6-12 indicated high expression, whereas scores of 0-4 represented low expression.

2.3 | Cell lines and cell culture

A human peritoneal mesothelial cell (HPMC) line, which was established by Prof. Ronco, was kindly provided by Prof. You-Ming Peng (Second Hospital of Zhongnan University, Changsha, China). HPMCs were cultured in RPMI 1640 medium containing 10% fetal bovine serum (FBS). Human GC cell lines MKN-45, MKN-28, SGC-7901, MGC-803, and BGC-823 were purchased from the Cell Bank of Type Culture Collection of Chinese Academy of Sciences (Shanghai, China). Human GC cells were cultured in RPMI 1640 medium or DMEM supplemented with 10% FBS. All cell cultures were incubated continuously under 5% CO2 at 37°C. For the GC and HPMC coculture system, transwell chambers (0.4-μm pores, Corning) separated by a polycarbonate membrane were used similar to the previous experiments performed in our laboratory. GC cells (5.0 × 105 cells) were seeded into the top chamber, and HPMCs (1.0 × 105 cells) were placed in the bottom chamber. GC cells had no direct contact with HPMCs, but the soluble factors derived from the GC cell lines could reach HPMCs.
2.4 | Transfection

TGF-β1-RNAi and SPHK1-RNAi lentiviruses were constructed by GeneChem (Shanghai, China). GC cells or HPMCs were infected with lentiviral particles in the presence of polybrene according to the manufacturer’s instructions. Infected cells were selected using 5 μg/ml puromycin (Sigma, USA). Transfection with Flag-SPHK1 and negative-control plasmids (GeneChem, Shanghai, China) was performed using Lipofectamine 3000.

2.5 | Immunoblot analysis

Western blotting was performed as previously described. Briefly, cells were harvested, lysed, and centrifuged. Equal amounts of proteins were separated by SDS-PAGE and transferred onto PVDF membranes (Millipore). The membranes were blocked in 5% nonfat milk and incubated with primary antibodies for TGF-β1, SPHK1, E-cadherin, N-cadherin, α-SMA, and GAPDH (Abcam) at 4°C overnight. After incubation in the corresponding secondary antibodies, membranes were incubated in the enhanced chemiluminescence solution (Thermo Fisher Scientific).

2.6 | Enzyme-linked immunosorbent assay (ELISA)

TGF-β1 levels in the culture supernatant of GC cells were measured using the Human TGF-β1 Immunoassay Kit (R&D Systems) as described previously.

2.7 | Confocal microscopy

Human peritoneal mesothelial cells were transfected with recombinant mRFP-GFP-LC3 adenovirus (Hanbio Biotechnology, Shanghai, China) as described previously. In green- and red-merged images, autophagosomes are shown as yellow puncta, whereas autolysosomes are shown as red puncta. In addition, autophagic flux was detected using a scanning confocal microscope.

2.8 | Transmission electron microscopy

As described previously, HPMCs were fixed with 2.5% glutaraldehyde in 0.1 mol L⁻¹ sodium cacodylate buffer, stored at 4°C overnight, and postfixed with 1% OsO₄ for 1.5 hours. After staining with 3% aqueous uranyl acetate and dehydration in a graded ethanol series, cells were embedded in Epon resin. Ultrathin sections were examined using a transmission electron microscope.

2.9 | Adhesion and invasion assay

The adhesion and invasion of GC cells in the presence of HPMCs were determined as described previously. For the adhesion assay, HPMCs (precedingly cocultured with GC) were plated in 96-well plates. GC cells were incubated with 5 μmol L⁻¹ Calcein-AM (Sigma, USA) and added to HPMCs, and incubated for 1 hour. Afterward, the plates were washed three times with PBS to remove nonadherent GC cells. The cell number was counted under a fluorescence microscope. For the invasion assay, HPMCs cocultured with GC cells were seeded into the upper chamber of 24-well transwell inserts (8-µm pore size; Corning) that were coated with 100 µL of Matrigel. When the HPMCs reached 90% confluence, 2 × 10⁵ GC cells were resuspended in 100 µL of serum-free medium and added into the upper chamber. The lower chamber was filled with 600 µL of DMEM containing 10% FBS as a chemoattractant. After incubation for the indicated times, cells on the lower surface were fixed and stained with 4% trypan blue. The percentage of stained cells was calculated under a microscope using five different fields of view.

2.10 | In vivo GCPD assay

A xenograft model of cancer cells mixed with stromal cells was established as previously reported. Five-week-old male BALB/c nude mice (Beijing Vital River, China) were used. SGC-7901 cells (2 × 10⁵) were mixed with HPMCs (5 × 10⁵) and implanted into the abdominal cavities of mice (n = 10). Mice were killed 6 weeks after implantation. Then, the locations of macroscopic tumors were recorded, and the tumor nodules were removed and weighed. All animal experimental procedures were approved by the Animal Research Committee of China Medical University.

2.11 | Statistical analysis

A two-tailed Student’s t test was used to calculate average differences between groups. The Kaplan-Meier method was used to conduct survival curves. Associations between different variables and overall survival were performed with the Cox proportional hazards regression model; hazard ratios (HRs) and 95% CIs were reported. The correlation between SPHK1 expression and clinicopathological factors in GC was calculated using the chi-squared test. All statistical analyses were conducted using SPSS 21.0, and P < 0.05 was considered statistically significant.

3 | RESULTS

3.1 | SPHK1 upregulation in the peritoneum is correlated with LC3B expression, peritoneal recurrence, and poor survival in GC

We first investigated SPHK1 and LC3B expression in peritoneal tissues from 120 patients with GC. IHC analysis
revealed that the SPHK1 and LC3B-positive expression rates were 36.7 (44/120) and 45.0% (54/120), respectively (Figure 1A). High-resolution images were included in the supplementary Figures S1–S4. In addition, SPHK1 expression was positively correlated with LC3B expression in peritoneal tissues (Pearson's coefficient test, $r = 0.456$; $P < 0.001$; Figure 1B). We further analyzed the association of SPHK1 with clinicopathological characteristics and prognosis in these patients. High SPHK1 expression was significantly associated with larger tumor size, deeper depth of tumor invasion, lymph node metastasis, advanced TNM stage, high LC3B expression, and peritoneal recurrence (Table 1). Conversely, there was no correlation of SPHK1 expression with gender, age, and tumor differentiation. Kaplan-Meier analysis illustrated that patients with high SPHK1 and LC3B expression had poor OS ($P_{\text{SPHK1}} < 0.001$, $P_{\text{LC3B}} = 0.009$, Figure 1C and D). After adjustment for potential confounding factors, multivariate Cox regression analysis identified SPHK1 upregulation as an independent factor for OS ($P = 0.031$, Table 2).

**FIGURE 1** Upregulated SPHK1 in peritoneum is correlated with LC3B and poor survival in GC. (A) Representative immunohistochemistry (IHC) staining with SPHK1 and LC3B in GC peritoneum tissues. (B) Scatter plots showing the positive correlation between SPHK1 and LC3B IHC scores in peritoneum tissues. (C) Kaplan-Meier survival curves based on SPHK1. (D) Kaplan-Meier survival curves based on LC3B.
3.2 | GC cell line SGC-7901 upregulates SPHK1 expression in HPMCs and induced HPMC autophagy via TGF-β1

Considering that TGF-β1 is an important paracrine protein, we first investigated TGF-β1 levels in intracellular and in culture supernatants in GC cell lines via western blotting and ELISA. Consistently, SGC-7901 cells presented highest levels of TGF-β1 in intracellular and in culture medium (Figure 2A and B). Thus, SGC-7901 cells were selected for use in the coculture model for subsequent experiments. Then, SGC-7901 cells were transfected with shRNAs. TGF-β1 expression was efficiently suppressed, as determined via western blotting and ELISA (Figure 2C and D).

3.3 | HPMCs autophagy stimulates GC cell adhesion and invasion

To determine the role of HPMCs autophagy in the development of GCPD, cocultured HPMCs were treated with the autophagy inhibitor 3-methyladenine (3-MA) at a concentration of 5 mmol L⁻¹. Figure 3A shows that SGC-7901-induced autophagy was significantly reduced in 3-MA-treated cells. Then, we performed adhesion and invasion assays for GC cells in peritoneal coculture models with HPMCs. By fluorescently examining the numbers of SGC-7901 and MGC-803 cells adhering to HPMCs, we found that the attachment of GC cells was significantly decreased for SGC-7901-shTGF-β1-cocultured and 3-MA-treated HPMCs compared with the findings for SGC-7901-shCtrl-cocultured cells (Figure 3B). In the GC cell-HPMC invasion assays, the SGC-7901-shTGF-β1-cocultured and 3-MA-treated HPMC monolayer barriers were less vulnerable to damage by GC cells (Figure 3C). These results suggested that TGF-β1-induced HPMC autophagy promoted the adhesion and invasion of GC cells.

3.4 | SPHK1 is required for TGF-β1-induced HPMCs autophagy and GCPD promotion

Next, we tried to confirm the role of SPHK1 in TGF-β1-induced HPMC autophagy. We depleted SPHK1 expression in HPMCs using SPHK1 small interfering RNA (Figure 4A). Then, HPMCs were cocultured with SGC-7901-shCtrl and shTGF-β1 cells. Western blotting revealed that SGC-7901-cocultured HPMCs transfected with SPHK1 shRNA exhibited decreased LC3 lipidation as well as p62/SQSTM1 degradation compared with the findings in shCtrl-transfected cells (Figure 4B). Figure 4C obviously revealed that the numbers of autophagosomes and autophagolysosomes were decreased in SPHK1-depleted HPMCs. As a gold standard for...
autophagosome detection, transmission electron microscopy revealed that autophagy-induced TGF-β1 paracrine signaling was significantly reduced by SPHK1 shRNA (Figure 4D).

We further examined the effects of SPHK1 expression in HPMCs on the adhesion and invasion of GC cells. The attachment and invasion of SGC-7901 and MGC-803 cells to shSPHK1 HPMC monolayers was significantly inhibited (Figure 5A and B). To investigate the interactions between HPMCs and GC cells in GCPD in vivo, we created a mouse model. SGC-7901 cells were injected intraperitoneally into BALB/c nude mice (n = 5 for each group) admixed with shSPHK1 or shCtrl HPMCs. The results demonstrated that SGC-7901 cells coinjected with shSPHK1 HPMCs exhibited reduced macroscopic nodules during peritoneal cavity dissemination to the mesentery, greater omentum, and parietal peritoneum (Figure 5C). In addition, the SGC-7901/shSPHK1-HPMC tumor weight was significantly lower than that of the matched tumors in which SPHK1 expression was not suppressed (Figure 5C).

3.5 | SPHK1 regulates HPMCs fibrosis by promoting autophagy

Previously, we reported that TGF-β1 induced peritoneal fibrosis and promoted GCPD.6,7 Thus, we investigated whether SPHK1 mediated TGF-β1-induced peritoneal fibrosis. In the coculture system of GC cells and HPMCs, we detected the expression of epithelial and mesenchymal proteins in HPMCs by western blotting. The increased expression of E-cadherin and decreased expression of N-cadherin and α-SMA suggested that TGF-β1 downregulation in SGC-7901 cells significantly weakened TGF-β1-induced HPMC fibrosis (Figure 6A). Moreover, the fibroblastic phenotype was substantially attenuated in shSPHK1 HPMCs under TGF-β1 paracrine action.

To further confirm the effects of SPHK1 on HPMCs autophagy and fibrosis, we overexpressed SPHK1 via Flag-SPHK1 plasmid transfection. SPHK1 overexpression resulted in increased LC3-II expression and decreased p62/SQSTM1 expression (Figure 6B). Meanwhile, E-cadherin downregulation and N-cadherin and α-SMA upregulation were also observed in Flag-SPHK1 HPMCs, indicating that SPHK1 induced fibrosis in HPMCs. Studies have indicated that autophagy is a regulator of fibrogenesis in some tissues and cells.27,28 Hence, we explored the regulation of fibrosis by SPHK1-induced autophagy. We treated SPHK1-overexpressing HPMCs with 5 mmol L⁻¹ 3-MA. The results demonstrated that autophagy was decreased in HPMCs. Moreover, the upregulation of N-cadherin and α-SMA and

| Parameters                      | Univariate analysis | Multivariate analysis |
|--------------------------------|---------------------|-----------------------|
|                                | HR (95% CI)         | P-value               |
| Age (≥65 years)                | 0.532 (0.335-0.845) | 0.007                 |
| Gender (male)                  | 0.827 (0.494-1.383) | 0.469                 |
| Tumor size (≥5 cm)             | 1.474 (0.926-2.345) | 0.102                 |
| Differentiation (poor)         | 0.804 (0.505-1.280) | 0.358                 |
| Depth of tumor invasion (T1-T4)| 1.752 (1.351-2.272) | <0.001                |
| Lymph node metastasis (+)      | 1.508 (1.272-1.786) | <0.001                |
| Peritoneal recurrence (+)      | 4.240 (2.622-6.857) | <0.001                |
| High SPHK1 expression          | 3.114 (1.950-4.975) | <0.001                |
| High LC3B expression           | 1.824 (1.147-2.899) | 0.011                 |

HR, hazard ratio; CI, confidence interval.

TABLE 2 Univariate and multivariate Cox proportional hazards analyses on overall survival for gastric cancer patients

FIGURE 2 GC cell line SGC-7901 upregulated HPMCs SPHK1 expression and induced HPMCs autophagy via TGF-β1. (A) TGF-β1 expression in five GC cell lines detected by western blotting. (B) TGF-β1 levels in condition medium of five GC cell lines analyzed by ELISA. (C) Protein expression of TGF-β1 after transfection with shTGF-β1 lentivirus in SGC-7901. (D) TGF-β1 levels in condition medium of SGC-7901 transected with shRNA lentivirus. (E) Western blot showing the expression of LC3B, P62/SQSTM1, and SPHK1 in HPMCs cocultured with SGC-7901 after 24, 48, and 72 hour. (F) The effect of TGF-β1 receptor inhibitor SB431542 on LC3B, P62/SQSTM1, and SPHK1 expression in HPMCs. (G) Immunofluorescent micrographs demonstrating mRFP-GFP-LC3 fusion protein in HPMCs cocultured with SGC-7901 after 48 hour. *P < 0.05
downregulation of E-cadherin were reversed. Taken together, these data suggested that SPHK1‐induced autophagy participates in regulating HPMCs fibrogenesis (Figure 6C).

4 | DISCUSSION

Peritoneal dissemination has been recognized as the most common form of metastasis in advanced GC as well as the leading cause of death in patients with GC.2,3 Many efforts have been made in clinical research, but the results are unsatisfactory. The mechanism of GCPD needs further investigation.

The hypothesis of “seed and soil” has been widely accepted, which suggests that the interaction between cancer cells and the peritoneal microenvironment leads to PD.29 A completely confluent mesothelial layer is the first barrier against bacterial invasion and tumor attachment.30
Our previous results demonstrated that intraperitoneal exfoliated GC cells induced senescence and apoptosis in HPMCs, leading to exposure of the subcutaneous matrix and forming a favorable apterium for GC cell implantation. This injury of HPMCs was mainly mediated by TGF-β1. Meanwhile, paracrine TGF-β1 from GC cells was required for TGF-β1-induced HPMC autophagy. (A) Western blotting detected SPHK1 expression after transfection with shSPHK1 lentivirus in HPMCs. (B-D) HPMCs were transfected with shCtrl or shSPHK1 and cocultured with SGC-7901 cells with existence or inhibition of TGF-β1. (B) Western blotting analyzed the LC3B and P62/SQSTM1 expression in HPMCs. (C) Immunofluorescent micrographs and (D) transmission electron microscopy showed autophagosomes in HPMCs.
induced HPMCs fibrosis, which increased the adhesion of GC cells to the peritoneum and endowed GC cells with enhanced invasiveness.\(^7\)

TGF-\(\beta\)1 is a member of the cytokine family that has been implicated in cell growth, differentiation, and apoptosis, and it is considered a crucial regulator of fibrosis.\(^32\) Functionally, TGF-\(\beta\)1-mediated regulation of autophagy has recently been reported in physiological and disease conditions.\(^33\) In the present study, we used GC cell/HPMC coculture systems and found that autophagy was activated in HPMCs by TGF-\(\beta\)1 released from GC cells. This phenomenon was reduced in the presence of TGF-\(\beta\)1 receptor inhibitor. Meanwhile, we found that TGF-\(\beta\)1-induced HPMCs autophagy promoted GC cell adhesion and invasion.

TGF-\(\beta\)1 regulates autophagy in different cell types through multiple signaling pathways. It was reported that continuous TGF-\(\beta\) exposure induced breast cancer cell autophagy and proteolytic degradation of Disabled-2.\(^34\) This process was mediated by cathepsin B. In human cardiac fibroblasts, TGF-\(\beta\)1 promotes fibrogenesis and autophagic activation.\(^35\) In the present study, SPHK1 was upregulated by TGF-\(\beta\)1 treatment, and the pivotal role of SPHK1 in TGF-\(\beta\)1-induced HPMCs

**FIGURE 5** SPHK1 expression in HPMCs regulated GC cells adhesion, invasion, and GCPD. (A, B) HPMCs were transfected with shCtrl or shSPHK1 and cocultured with SGC-7901 cells with existence or inhibition of TGF-\(\beta\)1. Adhesion (A) and invasion (B) of SGC-7901 and MGC-803 cells to the HPMCs. (C) Representative tumor nodules in the abdominal cavity of nude mice that were intraperitoneally injected with SGC-7901 cells admixed with shSPHK1 and shCtrl HPMCs, respectively. *\(P\) < 0.05
autophagy was confirmed by depleting SPHK1 in HPMCs. However, the precise molecular mechanism by which SPHK1 regulates HPMC autophagy is currently unclear, and we will conduct further investigation in the future.

In the current study, we first discovered that high SPHK1 expression in HPMCs was correlated with LC3B expression, peritoneal recurrence, and poor prognosis in patients with GC. TGF-β1-driven HPMCs autophagy promoted the adhesion and invasion of GC cells by regulating SPHK1 in vitro, thus stimulating GCPD in vivo. Our previous studies identified the central role of HPMCs fibrosis in GCPD. Additionally, studies have suggested that SPHK1 is activated in liver and renal tubular fibrosis. Thus, we hypothesized that SPHK1-mediated autophagy might be a regulator of HPMCs fibrosis. The results illustrated that HPMC fibrosis was substantially reduced in shSPHK1 HPMCs under the paracrine action of TGF-β1. Consistently, SPHK1 over-expression was associated with increased fibrosis and autophagy. Interestingly, HPMCs fibrosis was reduced in the presence of an autophagy inhibitor. These results suggested that SPHK1-mediated autophagy instigates TGF-β1-induced HPMCs fibrosis, which might be the mechanism by which GCPD is promoted (Figure 6C).

In conclusion, this study demonstrated that TGF-β1 induces autophagy in HPMCs and promotes GCPD through SPHK1. SPHK1-mediated autophagy might be a regulator of HPMC fibrosis. Our results provided new data for understanding the mechanisms of GCPD and established SPHK1 as a novel target for controlling GCPD.

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CONFLICT OF INTEREST

The authors declare no conflict of interests.
REFERENCES

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68:394-424.

2. Van Cutsem E, Sagar X, Topal B, Haustermans K, Prenen H. Gastric cancer. Lancet. 2016;388:2654-2664.

3. Thomassen I, van Gestel YR, van Ramshorst B, et al. Peritoneal carcinomatosis of gastric origin: a population-based study on incidence, survival and risk factors. Int J Cancer. 2014;134:622-628.

4. Nashimoto A, Akazawa K, Isobe Y, et al. Gastric cancer treated in 2002 in Japan: 2009 annual report of the JGCA nationwide registry. Gastric Cancer. 2013;16:1-27.

5. Lv ZD, Zhao WJ, Jin LY, et al. Blocking TGF-beta1 by P17 peptides attenuates gastric cancer cell induced peritoneal fibrosis and prevents peritoneal dissemination in vitro and in vivo. Biomed Pharmacother. 2017;88:27-33.

6. Miao ZF, Zhao TT, Wang ZN, et al. Transforming growth factor-beta1 signaling blockade attenuates gastric cancer cell-induced peritoneal mesothelial cell fibrosis and alleviates peritoneal dissemination both in vitro and in vivo. Tumour Biol. 2014;35:3575-3583.

7. Lv ZD, Na D, Liu FN, et al. Induction of gastric cancer cell adhesion through transforming growth factor-beta1-mediated peritoneal fibrosis. J Exp Clin Cancer Res. 2010;29:139.

8. Mizushima N. A brief history of autophagy from cell biology to physiology and disease. Nat Cell Biol. 2018;20:521-527.

9. Giampieri F, Afrin S, Forbes-Hernandez TY, et al. Autophagy in Human health and disease: novel therapeutic opportunities. Antioxid Redox Signal. 2018;30:577-634.

10. Singh KK, Lovren F, Pan Y, et al. The essential autophagy gene ATG7 modulates organ fibrosis via regulation of endothelial-to-mesenchymal transition. J Biol Chem. 2015;290:2547-2559.

11. Koesters R, Kassling B, Lehir M, et al. Tubular overexpression of transforming growth factor-beta1 induces autophagy and fibrosis but not mesenchymal transition of renal epithelial cells. Am J Pathol. 2010;177:632-643.

12. Patel AS, Lin L, Geyer A, et al. Autophagy in idiopathic pulmonary fibrosis. PLoS ONE. 2012;7:e41394.

13. Ogretmen B. Sphingolipid metabolism in cancer signalling and therapy. Nat Rev Cancer. 2018;18:33-50.

14. Sukocheva OA. Expansion of sphingosine kinase and sphingosine-1-phosphate receptor function in normal and cancer cells: from membrane restructuring to mediation of estrogen signaling and stem cell programming. Int J Mol Sci. 2018;19:1-2.

15. Datta A, Loo SY, Huang B, et al. SPHK1 regulates proliferation and survival responses in triple-negative breast cancer. Oncotarget. 2014;5:5920-5933.

16. Matula K, Collie-Duguid E, Murray G, et al. Regulation of cellular sphingosine-1-phosphate by sphingosine kinase 1 and sphingosine-1-phosphate lyase determines chemotheraphy resistance in gastroesophageal cancer. BMC Cancer. 2015;15:762.

17. Moruno Manchon JP, Uzor NE, Finkbeiner S, Tsvetkov AS. SPHK1/sphingosine kinase 1-mediated autophagy differs between neurons and SH-SY5Y neuroblastoma cells. Autophagy. 2016;12:1418-1424.

18. Miao ZF, Wang ZN, Zhao TT, et al. Peritoneal milky spots serve as a hypoxic niche and favor gastric cancer stem/progenitor cell peritoneal dissemination through hypoxia-inducible factor 1alpha. Stem Cells. 2014;32:3062-3074.

19. Zhu YP, Sheng LL, Wu J, et al. Loss of ARID1A expression is associated with poor prognosis in patients with gastric cancer. Hum Pathol. 2018;78:28-35.

20. Miao ZF, Xu H, Xu HM, et al. DLL4 overexpression increases gastric cancer stem/progenitor cell self-renewal ability and correlates with poor clinical outcome via Notch-1 signaling pathway activation. Cancer Med. 2017;6:245-257.

21. Rougier JP, Moullier P, Piedagnel R, Ronco PM. Hyperosmolality suppresses but TGF beta 1 increases MMP9 in human peritoneal mesothelial cells. Kidney Int. 1997;51:337-347.

22. Miao ZF, Wu JH, Wang ZN, et al. Endoglin overexpression mediates gastric cancer peritoneal dissemination by inducing mesothelial cell senescence. Hum Pathol. 2016;51:114-123.

23. Sahoo N, Collard TJ, Southern SL, et al. BAG-1 suppresses expression of the key regulatory cytokine transforming growth factor beta (TGF-beta1) in colorectal tumour cells. Oncogene. 2013;32:4490-4499.

24. Lan T, Li C, Yang G, et al. Sphingosine kinase 1 promotes liver fibrosis by preventing miR-19b-3p-mediated inhibition of CCR2. Hepatology. 2018;68:1070-1086.

25. Schmeisser H, Fey SB, Horowitz J, et al. Type I interferons induce autophagy in certain human cancer cell lines. Autophagy. 2013;9:683-696.

26. Cai J, Tang H, Xu L, et al. Fibroblasts in omentum activated by tumor cells promote ovarian cancer growth, adhesion and invasiveness. Carcinogenesis. 2012;33:20-29.

27. Gharavi M, Cunnington RH, Gupta S, et al. Autophagy is a regulator of TGF-beta1-induced fibrogenesis in primary human atrial myofibroblasts. Cell Death Dis. 2015;6:e1696.

28. Livingston MJ, Ding HF, Huang S, Hill JA, Dong Z. Persistent activation of autophagy in kidney tubular cells promotes renal interstitial fibrosis during unilateral ureteral obstruction. Autophagy. 2016;12:976-998.

29. Paget S. The distribution of secondary growths in cancer of the breast. 1889. Cancer Metastasis Rev. 1989;8:98-101.

30. Mutsaers SE, Wilkosz S. Structure and function of mesothelial cells. Cancer Treat Res. 2007;134:1-19.

31. Na D, Lv ZD, Liu FN, et al. Gastric cancer cell supernatant causes gastric cancer peritoneal dissemination by inducing mesothelial cell senescence. Cancer Med. 2017;6:2463-2473.

32. Suzuki HI, Kiyono K, Miyazono K. Regulation of autophagy by transforming growth factor-beta signaling. Autophagy. 2010;6:645-647.

33. Kiyono K, Suzuki HI, Matsuyama H, et al. Autophagy is activated by TGF-beta and potentiates TGF-beta-mediated growth inhibition in human hepatocellular carcinoma cells. Cancer Res. 2009;69:8844-8852.

34. Jiang Y, Woosley AN, Sivalingam N, Natarajan S, Howe PH. Cathepsin-B-mediated cleavage of Disabled-2 regulates TGF-beta-induced autophagy. Nat Cell Biol. 2016;18:851-863.

35. Zou M, Wang F, Gao R, et al. Autophagy inhibition of hsa-miR-19a-3p/19b-3p by targeting TGF-beta R II during TGF-beta1-induced fibrogenesis in human cardiac fibroblasts. Sci Rep. 2016;6:24747.
36. Da C, Ren Y, Yao F, et al. Sphingosine kinase 1 protects renal tubular epithelial cells from renal fibrosis via induction of autophagy. *Int J Biochem Cell Biol*. 2017;90:17-28.

**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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