Activation of GLP-1 receptor enhances the chemosensitivity of pancreatic cancer cells

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Abstract

This study aimed to determine whether and how the glucagon-like peptide 1 receptor (GLP-1R) agonist liraglutide affects the chemoresistance and chemosensitivity of pancreatic cancer cells to gemcitabine *in vitro* and *in vivo*. The GLP-1R and protein kinase A (PKA) levels were compared between the human pancreatic cancer cell line PANC-1 and the gemcitabine-resistant cell line PANC-GR. The *in vitro* effects of liraglutide on the cell proliferation and apoptosis as well as the nuclear factor-kappa B (NF-κB) expression levels of PANC-GR cells were evaluated. In addition, a mouse xenograft model of human pancreatic cancer was established by s.c. injection of PANC-1 cells, and the effects of liraglutide on the chemosensitivity were evaluated *in vitro* and *in vivo*. In contrast to PANC-1 cells, PANC-GR cells exhibited lower expression levels of GLP-1R and PKA. Incubation with liraglutide dose dependently inhibited the growth, promoted the apoptosis, and increased the expression of GLP-1R and PKA of PANC-GR cells. Similar effects of liraglutide were observed in another human pancreatic cancer cell line MiaPaCa-2/MiaPaCa-2-GR. Either the GLP-1R antagonist Ex-9, the PKA inhibitor H89, or the NF-κB activator lipopolysaccharide (LPS) could abolish the antiproliferative and proapoptotic activities of liraglutide. Additionally, each of these agents could reverse the expression of NF-κB and ABCG2, which was decreased by liraglutide treatment. Furthermore, liraglutide treatment increased the chemosensitivity of pancreatic cancer cells to gemcitabine, as evidenced by *in vitro* and *in vivo* experiments. Thus, GLP-1R agonists are safe and beneficial for patients complicated with pancreatic cancer and diabetes, especially for gemcitabine-resistant pancreatic cancer.

Introduction

Pancreatic cancer is closely related to diabetes mellitus. Diabetes mellitus is not only a risk factor for pancreatic cancer, but it is also one of the clinical manifestations of pancreatic cancer (Everhart & Wright 1995, Chari et al. 2008). It is believed that glycemic control affects the prognosis of patients with pancreatic cancer and that long-term hyperglycemia is a poor prognostic factor (Lee et al. 2016). Therefore, optimization of the selection of hypoglycemic drugs in patients complicated with pancreatic cancer and diabetes mellitus has important
GLP-1R activation inhibits pancreatic cancer. Although several genetic and/or epigenetic alterations have been found to contribute to the chemoresistance of pancreatic adenocarcinomas ([Kim & Gallick 2008, Tamburrino et al. 2013]), the potential mechanisms involved in gemcitabine resistance still lack clear explanations. Thus, understanding the molecular mechanisms of drug resistance in pancreatic cancer as well as seeking high-efficiency, low-toxic new chemotherapy sensitizers are critical to develop new effective treatments for this deadly disease.

ATP-binding cassette subfamily G member 2 (ABCG2), commonly referred to as breast cancer resistance protein (BCRP), is a 655-amino-acid polypeptide transporter with a wide range of substrates that displays resistance to a variety of drugs, including gemcitabine ([Mo & Zhang 2012, Natarajan et al. 2012]). Upregulation of BCRP/ABCG2 has been reported to play a role in chemoresistance via the PI3K/Akt and NF-κB signaling pathways in breast cancer ([Zhang et al. 2011]). In another study, modulation of ABCG2 expression in pancreatic cancer cells inhibited cell migration and invasion, but no effects on cell proliferation or apoptosis were observed ([Wang et al. 2010]).

The present study aimed to further elucidate the effects of the GLP-1R agonist liraglutide on pancreatic cancer from the perspective of chemoresistance and chemosensitivity, so as to provide a new therapeutic strategy for pancreatic cancer patients with diabetes mellitus. We investigated whether and how activation of GLP-1R by liraglutide affects the proliferation and apoptosis of gemcitabine-resistant human pancreatic cancer cells. Based on previous work by us and others, we hypothesized that there would be an association between GLP-1R activation and chemosensitivity in pancreatic cancer cells, probably by regulation of NF-κB/ABCG2. In addition, we also explored the role of liraglutide on the chemosensitivity of pancreatic cancer cells to gemcitabine.

Materials and methods

Cell culture

The human pancreatic cancer cell lines PANC-1 and MiaPaCa-2 were purchased from the American Type Culture Collection. The gemcitabine-resistant human pancreatic cancer cell line PANC-GR was established by subjecting PANC-1 cells to gradually increasing, low concentrations of gemcitabine. Briefly, the median lethal dose of gemcitabine for PANC-1 cells was evaluated after a week of intervention with different concentrations of gemcitabine and was determined to be 0.08 μg/mL. Initially, the PANC-1 cells were cultured in 0.1 μg/mL gemcitabine for 48 h with a defined drug-free interval. When the cells adapted to the dose, they were cultured in medium containing 0.4 μg/mL gemcitabine. The culture was continued with a four-fold increasing concentration of gemcitabine until it reached 400 μg/mL. Finally, cells stably resistant to a high concentration of gemcitabine were obtained. Cells were cultured in Dulbecco’s modified Eagle’s medium (Life Technologies) supplemented with 10% fetal bovine serum at 37°C and 5% CO2 in a humidified incubator. The MiaPaCa-2-GR cell line was established with similar methods as described earlier, with the median lethal dose of gemcitabine for MiaPaCa-2 cells determined to be 0.16 μg/mL.

Cell proliferation assay

Cells were seeded in 96-well plates at a density of 3 x 10^3/well. After serum starvation for 12 h, the cells were...
incubated with liraglutide (NovoNordisk, Bagsvaerd, Denmark) at 0, 10, 100, and 1000 nM for 48 h.

For the in vitro experiments determining the signaling pathways involved in cell proliferation, the cells were first serum-starved for 12 h, followed by incubation with liraglutide (100 nM), the GLP-1R antagonist Ex-9 (500 nM; Sigma), the protein kinase A (PKA) inhibitor H89 (10 μM; Cell Signaling Technology), the NF-κB activator lipopolysaccharide (LPS) (100 ng/mL; Enzo Life Sciences International, Farmingdale, NY, USA), or their combination for 48 h.

To determine whether there was a synergistic antiproliferative effect between liraglutide and gemcitabine, the cells were first exposed to serum starvation for 12 h and then were incubated with liraglutide (100 nM), gemcitabine (0.2 μM; Lilly, France), or their combination for 48 h.

The Cell Counting Kit-8 (CCK-8, Kumamoto, Japan) assay was used to determine the number of viable cells under each treatment condition at the end of the incubation period. The absorbance at 450 nm (optical density value) in each well was measured by a spectrometer (Thermo Scientific). A standard growth curve was constructed by using serial dilutions of a known template. The number of cells in each well was then calculated according to the standard curve.

**Colony formation assay**

To assess whether the activation of GLP-1R affected the proliferation of PANC-GR cells, we evaluated colony formation by seeding cells at a low plating density. PANC-GR cells were harvested and prepared into a single-cell suspension, and then they were seeded onto six-well plates at a density of $3 \times 10^4$. The cells were continuously cultured for 14 days in the absence or presence of liraglutide (0, 10, 100, or 1000 nM). At the end of the culture, the colonies were fixed and stained with crystal violet (Sigma).

**Cyclic adenosine monophosphate (cAMP) measurement**

After counting the cells, the cells were lysed and then repeatedly frozen and thawed four times. The supernatant was collected after centrifugation. The level of cAMP in the supernatant was measured by an ELISA with a commercially available kit (R&D Systems). The optical density, which was proportional to the cAMP concentration, was determined by using a microplate reader at a wavelength of 450 nm (Thermo Scientific). Calculation of the cAMP concentration was performed by reference to a standard curve constructed with the cAMP standards provided in the kit.

**Reverse transcription–polymerase chain reaction (RT-PCR)**

Total RNA was extracted from the cells by using TRIzol reagent (Invitrogen) and an RNeasy Mini Kit (Qiagen). RNA samples were reverse-transcribed into cDNA using a First Strand cDNA Synthesis Kit (Thermo Scientific). The primer sequences used are shown in Table 1. DNA amplification was performed on a GeneAmp® PCR System 9700 thermal cycler (Applied Biosystems) with Taq PCR Master Mix (Promega). The PCR products were visualized by agarose gel electrophoresis, and the optical density of the gene of interest was analyzed by correcting with the control (GAPDH).

**Western blot**

Cells were lysed, and the proteins in the supernatant were quantified. Samples (20–80 μg of target protein) were separated by SDS–PAGE and transferred to a nitrocellulose membrane. The membranes were incubated overnight at 4°C in a solution containing one of the following primary antibodies: rabbit anti-human GLP-1R (Abcam), PKA (Cell Signaling Technology), NF-κB (Santa Cruz Biotechnology), ABCG2 (Calbiochem, San Diego, CA, USA), Bax (Cell Signaling Technology), caspase-3 (Sigma) and mouse anti-human antibody GAPDH (Zhongshan Golden Bridge Biotechnology, Beijing, China). The membranes were incubated at room temperature for 1–2 h with IRDye800CW-conjugated goat anti-rabbit or goat anti-mouse IgG secondary antibody (dilution 1:10,000, LI-COR Biosciences, Lincoln, NE, USA). The band density was measured using an Odyssey CLx Infrared Imaging system (LI-COR Biosciences, USA).

**Flow cytometric analysis**

Apoptosis was determined by annexin V/propidium iodide (PI) staining, as described by the manufacturer (Roche Diagnostics). The harvested cells ($1.5 \times 10^6$) were stained with 5 μL of Annexin V-FITC and 5 μL of PI solution for 15 min in the dark and then analyzed by flow cytometry (BD Biosciences, San Jose, CA, USA). On the flow-cytometric figures, the proportion of cells in each quadrant was calculated as reported previously (Fan et al. 2013).
Table 1 Primer sequences used for RT-PCR.

<table>
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<tr>
<th>Genes</th>
<th>Forward</th>
<th>Reverse</th>
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<td>CACCCATCTTCTCTGCTTA</td>
<td>54</td>
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<td>GAATAGCCACCATCATAAAGG</td>
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<tr>
<td>GAPDH</td>
<td>ACAGTCAGCGCAGTCTCTTT</td>
<td>CTGGAAGATGGTGATGGGAT</td>
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<td>87</td>
</tr>
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Animals and tumor implantation experiments

All animal experiments were approved by the Peking University Animal Care and Use Committee. Five-week-old male athymic nude mice (Vital River Animal Center, Beijing, China) were used in this study. After adaption, the mice were subcutaneously injected with PANC-1 cells (1 × 10⁶ in 100 μL of PBS) in the right flank. Starting at 3 days after cell injection, the mice were intraperitoneally injected with 100 μL of PBS (vehicle), 0.2 mg/kg liraglutide, 80 mg/kg gemcitabine, or liraglutide + gemcitabine in 100 μL of PBS, twice daily for 4 weeks (n = 5 in each group). The weights of the mice were recorded. When the experiment was terminated, the mice were killed, the tumors were excised, and the weights and volumes of the tumors of each mouse were recorded. The tumor volume was calculated using the following equation: V = (a × b²) × 0.5236, where ‘a’ is the larger dimension and ‘b’ is the perpendicular diameter (Wolf et al. 2006). The tumor sections were paraffin-embedded and preserved for immunohistochemical analysis.

Immunohistochemical analysis

The tumor tissue was cut into a 5 × 5 × 1 mm³ volume, which was embedded in paraffin and cut into a thickness of 4–5 μm. The slides were blocked with nonimmune goat serum for 1 h and then incubated with primary antibody against rabbit anti-human Ki-67 (dilution, 1:200; Bioworld Technology, Dublin, OH, USA) or Bax (dilution, 1:200; Cell Signaling Technology) at 4°C overnight. After washing, the slides were incubated with biotinylated goat anti-rabbit IgG antibody as the secondary antibody (dilution, 1:100; Zhongshan Goldbridge Biotechnology, China). Positive immunostaining, which was visualized as yellow or brown granules in the cytoplasm and/or nuclei, was evaluated under a light microscope.

Statistical analysis

All statistical analyses were performed using SPSS11.5 software (SPSS Inc.). Data are presented as the mean ± S.D.
Apoptosis was determined with annexin V/PI staining using flow cytometry. Compared with the control exposed to 0 nM liraglutide, incubation with liraglutide for 48 h increased the apoptosis of cultured PANC-GR cells ($P<0.05$; Fig. 2F), and this effect was shown to occur in a dose-dependent manner. Consistently, the expression levels of apoptosis-associated proteins, including Bax and caspase-3, were significantly increased in cultured PANC-GR cells that were pretreated with liraglutide for 48 h, especially at a high concentration ($P<0.05$; Fig. 2G). We also found that pretreatment with liraglutide led to increased expression levels of Bax and caspase-3 in MiaPaCa-2-GR cells (Supplementary Fig. 1F and G).

**Liraglutide inhibits the growth and enhances the apoptosis of PANC-GR cells via GLP-1R/PKA-mediated downregulation of NF-κB and ABCG2**

We evaluated the difference in the expression levels of the transcriptional regulation factor NF-κB and the multidrug resistance gene product ABCG2 in PANC-GR cells versus PANC-1 cells. PANC-GR cells had significantly higher expression of NF-κB and ABCG2 as compared with PANC-1 cells, at both the mRNA and protein levels (Supplementary Fig. 2).

Next, we investigated the effects of liraglutide on NF-κB and ABCG2 expression. Incubation with liraglutide led to decreased expression of NF-κB and ABCG2, at both the mRNA and protein levels, in PANC-GR cells ($P<0.05$; Fig. 3A and B). In addition, these effects were shown to occur in a dose-dependent manner when the liraglutide concentration was 0 nM, 10 nM, 100 nM, or 1000 nM. Interestingly, treatment with the GLP-1R antagonist Ex-9 could block the effect of liraglutide on PKA, NF-κB, and ABCG2 expression, as detected by RT-PCR and Western blot (treatment with liraglutide + Ex-9 vs liraglutide alone, $P<0.05$; Fig. 3C and D). However, treatment with Ex9 alone did not affect the expression of PKA, NF-κB, or ABCG2 (treatment with Ex-9 alone vs control, $P>0.05$). Moreover, cell culture with liraglutide could significantly decrease the cell growth, although Ex-9 could reverse the inhibitory effect of liraglutide on the growth of PANC-GR cells (Fig. 3E). By annexin V/PI staining using flow cytometry, we found that liraglutide significantly promoted the apoptosis of PANC-GR cells ($P<0.05$ vs control), whereas Ex-9 could reduce liraglutide-induced apoptosis (treatment with liraglutide + Ex-9 vs liraglutide alone, $P<0.05$; Fig. 3F).

Similarly, treatment with either the PKA inhibitor H89 or the NF-κB activator LPS could effectively reverse...
the decreased expression of NF-κB and ABCG2 caused by liraglutide treatment (treatment with liraglutide+H89 or LPS vs liraglutide alone, $P<0.05$; Fig. 4A and B), almost to a level comparable to the control ($P>0.05$ vs control). Furthermore, treatment with either H89 or LPS could partially restore the in vitro growth of PANC-GR cells, which was inhibited by liraglutide (Fig. 4C). In addition, the proapoptotic effect of liraglutide was reduced by either H89 or LPS treatment (treatment with liraglutide+H89 or LPS vs liraglutide alone ($P<0.05$; Fig. 4D).

**Liraglutide enhanced the chemosensitivity of PANC-1 cells to gemcitabine**

We investigated whether liraglutide had synergistic antiproliferative and proapoptotic activities with gemcitabine. PANC-1 cells were treated with liraglutide, gemcitabine, or their combination for 48 h. We observed a further decrease of cell growth when the cells were exposed to gemcitabine+liraglutide as compared with gemcitabine alone ($P<0.05$, Fig. 5A). Additionally, liraglutide promoted the proapoptotic effect of gemcitabine, as evidenced by an increased proportion of apoptotic cells as well as enhanced expression of Bax and caspase-3 in PANC-1 cells (liraglutide+gemcitabine vs gemcitabine alone, $P<0.05$; Fig. 5B and C).

We further verified the synergistic effect of liraglutide in nude mice. Liraglutide treatment enhanced the chemosensitivity of pancreatic cancer cells to gemcitabine, as gemcitabine+liraglutide further reduced the tumor volume and tumor weight (Fig. 5D, E, F and G), as compared with gemcitabine alone. Also, the combination
of gemcitabine and liraglutide led to decreased Ki-67 but increased Bax immunoreactivity in tumor tissues by immunohistochemical analysis (Fig. 5H).

Discussion

Patients with pancreatic cancer have a high prevalence of diabetes mellitus (68%), and glycemic control affects the prognosis of patients with pancreatic cancer (Aggarwal et al. 2013). Therefore, it is of great clinical significance to optimize the selection of hypoglycemic drugs in patients with pancreatic cancer and diabetes mellitus. As a new hypoglycemic agent, GLP-1 has its own advantages, including a low risk of hypoglycemia and weight gain as well as cardiovascular and cerebrovascular protection (Vilsboll et al. 2007). In addition, studies have shown that GLP-1 or a GLP-1R agonist can inhibit a variety of tumor cells (Koehler et al. 2011, Ligumsky et al. 2012, Zhao et al. 2014b). For example, activation of GLP-1R by exendin-4, a GLP-1R agonist, inhibited the growth and increased the apoptosis of murine CT26 colon cancer cells via upregulating intracellular cAMP levels while inhibiting ERK1/2 in a PKA-dependent manner (Koehler et al. 2011). In another study, GLP-1 was found to be a potent inducer of cAMP, thus inhibiting breast cancer cell proliferation (Ligumsky et al. 2012). However, Koehler et al. found that GLP-1R activation by exendin-4 did not affect the growth and apoptosis of GLP-1R-expressing pancreatic cancer cells, although it could promote the production of cAMP and inhibit the phosphorylation of ERK1/2 (Koehler & Drucker 2006). We previously found that a lower GLP-1R expression in human pancreatic cancer tissues was associated with the growth, metastasis, and poor prognosis of patients (Zhao et al. 2014a). Also, activation of GLP-1R by liraglutide inhibits growth and promotes apoptosis of human pancreatic cancer cells in vitro, accompanied by cAMP production and resultant inhibition of the Akt and ERK1/2 signaling pathway (Zhao et al. 2014b).
In this study, for the first time, we investigated the expression and function of GLP-1R in gemcitabine-resistant human pancreatic cancer cells. Compared to PANC-1 cells, we found a lower GLP-1R expression in PANC-GR cells. Activation of GLP-1R by liraglutide inhibited the growth and promoted the apoptosis of PANC-GR cells in a dose-dependent manner, accompanied by decreased expression of NF-κB and ABCG2; these effects could be abolished by either the GLP-1R antagonist Ex-9, the PKA inhibitor H89, or the NF-κB activator LPS. Moreover, liraglutide increased the chemosensitivity of pancreatic cancer cells to gemcitabine, irrespective of in vitro or in vivo evidence. Together with our previous work (Zhao et al. 2014a, b), we provided data supporting that activation of GLP-1R has a direct antitumor effect against pancreatic cancer as well as synergistic antiproliferative and proapoptotic activity with gemcitabine.

GLP-1R is expressed in various tissues and organs of the human body. In addition, it has been reported that GLP-1R is expressed in a variety of endocrine tumors, but not in carcinomas or lymphomas (Korner et al. 2007). Our previous work showed that GLP-1R is expressed in normal pancreatic tissues; in contrast, pancreatic cancer tissues exhibit low or absent expression of GLP-1R. Furthermore, negative GLP-1R expression was more commonly observed in advanced pancreatic cancer with a larger diameter and/or lymphatic metastasis (Zhao et al. 2014a). This evidence suggests a possible relationship between GLP-1R and the malignant behaviors of pancreatic tissues. In the present study, we found a further decrease of GLP-1R expression in gemcitabine-resistant human pancreatic cancer cells, probably indicating the special role of GLP-1R in the onset and progression of pancreatic cancer. In this study, we further confirmed the antiproliferative and proapoptotic effects of liraglutide on gemcitabine-resistant human pancreatic cancer cells, likely related to upregulation of the GLP-1R/cAMP/PKA signaling pathway, which needs further investigation.

It is reported that GLP-1 exerts anti-inflammatory effects in chronic inflammatory diseases including type 2 diabetes by inhibiting the NF-κB signaling pathway (Lee & Jun 2016). In one study, liraglutide decreased NF-κB activation, which could be reversed by the PKA inhibitor H-89, indicating the involvement of the cAMP-PKA pathway in inhibition of NF-κB activation; liraglutide treatment subsequently led to the attenuation of ovalbumin-induced airway inflammation (Zhu et al. 2015). In fact, NF-κB is not only an important transcription factor in inflammation, immune response, cell proliferation, and apoptosis (Baker et al. 2011), but it also has been found to be a key regulator of tumor growth and chemotherapy resistance (Pramanik et al. 2018). Activation of NF-κB is frequently observed in pancreatic ductal adenocarcinoma, and an altered NF-κB signaling pathway contributes to therapeutic resistance (Prabhur et al. 2014). Silencing NF-κB may induce apoptosis and
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In this study, we found that GLP-1R activation with liraglutide decreased the expression of NF-κB, which could be abolished by the GLP-1R antagonist Ex-9, the PKA inhibitor H89, or the NF-κB activator LPS, consequently suppressing the growth and enhancing the apoptosis of gemcitabine-resistant human pancreatic cancer cells. Consistent with our results, Iwaya et al. reported that the GLP-1R agonist exendin-4 could attenuate the proliferation of breast cancer cells through activation of GLP-1R and subsequent inhibition of NF-κB activation (Iwaya et al. 2017). Taken together, GLP-1R agonists may have an antitumor effect in gemcitabine-resistant human pancreatic cancer cells, at least in part by inhibiting activation of the NF-κB signaling pathway.

Pancreatic cancer is a malignant tumor with a high mortality and a poor prognosis. Congenital or acquired drug resistance is the main reason for chemotherapy failure. Chemotherapeutic resistance is attributed to a variety of factors, among which drug efflux pumps mediated by ABC transporters is an important cause (Choi & Yu 2014). The ABCG2 protein is a member of the G subgroup of human ABC transporter proteins, and its gene is located on 4q22 of the human chromosome (Natarajan et al. 2012). ABCG2 has been found to confer the side population phenotype and therefore is considered as a marker of cancer stem cells (Ding et al. 2010). Studies have shown that the expression of ABCG2 is normally low or even absent in pancreatic tissues, but it is high in human pancreatic cancer cells (Wang et al. 2010, Skrypek et al. 2015). Further research has demonstrated that ABCG2 is a downstream gene of NF-κB, which can directly bind to the promoter region of the ABCG2 gene, thereby transcriptionally activating the expression of ABCG2 and subsequently contributing to the occurrence and development of tumors (Shojaie & Ghaffari 2016, Sui et al. 2016). However, the underlying mechanism has not been clearly elucidated. Gemcitabine has been reported to upregulate the expression of ABCG2/BCRP in pancreatic cancer cells, which initiates the onset of chemoresistance (Sun et al. 2016). In the present study, we found that PANC-GR cells had significantly higher expression of ABCG2 as compared with PANC-1 cells. Interestingly, GLP-1R activation with liraglutide led to decreased ABCG2 expression in PANC-GR cells and enhanced chemosensitivity of PANC-1 cells to gemcitabine, both in vitro and in vivo. These results suggest that GLP-1R activation not only suppresses tumor progression, but it may also attenuate the chemoresistance of pancreatic cancer cells to gemcitabine. Thus, our research provides a

Figure 5
Liraglutide enhances the chemosensitivity of pancreatic cancer cells to gemcitabine. (A, B and C) PAN-1 cells were incubated or cultured with GEM (0.2 μM), Lira (100 nM), or GEM (0.2 μM) + Lira (100 nM). (A) Cell proliferation was evaluated at 24 h and 48 h by using the Cell Counting Kit-8 assay. *p < 0.05 (GEM vs control); **p < 0.05 (Lira vs control); ***p < 0.05 (GEM + Lira vs control); *p < 0.05 (Lira + GEM vs GEM). (B) Flow cytometric quantification of apoptosis with annexin V and PI staining (left), and the percentage of apoptotic cells (right). (C) The expression levels of Bax and caspase-3 as detected by western blot (left), and their band intensities relative to β-actin (right). (D, E, F and G) Mouse xenograft models were established by subcutaneously injecting PANC-1 cells into the right flank of each nude mouse. (D) Representative images of tumors from mice treated with PBS, GEM, Lira, or GEM + Lira. The weight gains of mice (E), tumor volume (F), and tumor weights (G) were evaluated. The expression levels of Ki-67 and Bax in tumor tissue were determined by immunohistochemical analysis. Magnification, 100×. *p < 0.05 (vs control). Lira, liraglutide; GEM, gemcitabine. A full colour version of this figure is available at https://doi.org/10.1530/JME-19-0186.
scientific basis for elucidating the role and mechanism of the GLP-1R signaling pathway in NF-κB/ABCG2-mediated chemotherapeutic resistance as well as searching for molecular targets to reverse the chemotherapy resistance of pancreatic cancer.

In conclusion, these data provide the first evidence that liraglutide alone displays significant antitumor effects against gemcitabine-resistant human pancreatic cancer cells, and it further potentiates the therapeutic effectiveness of gemcitabine via modulation of the NF-κB signaling pathway and downstream ABCG2. Thus, GLP-1R agonists seem to be safe and beneficial for patients complicated with pancreatic cancer and diabetes, especially for gemcitabine-resistant pancreatic cancer. However, more research is still needed.

_Supplementary materials_  
This is linked to the online version of the paper at https://doi.org/10.1530/JME-19-0186.

_Declaration of interest_  
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

_Funding_  
This work was supported by grants from the National Natural Science Foundation of China (81602139, 81602403), the Natural Science Foundation of Tianjin City (16JCQNJC10100), and the Tianjin Hospital Science and Technology Foundation of Tianjin City (TJYY1508).

_Data availability_  
The datasets generated and analyzed during the present study are available from the corresponding author on reasonable request.

_Author contribution statement_  
H Z and Z R contributed to the conception and design of the research. L H, L Y, L D, and Y W performed the experiments. H Z prepared the figures. H Z and X J drafted the manuscript. L H edited and revised the manuscript. All authors approved the final version of the manuscript. Z R is the principal investigator of this work.

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Received in final form 23 October 2019
Accepted 17 December 2019
Accepted Manuscript published online 17 December 2019