Exenatide inhibits the growth of endometrial cancer Ishikawa xenografts in nude mice

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Abstract. Studies have showed that diabetes is one of the high risk factors of endometrial cancer; however, no reports describe the anti- or pro-cancer effect of a new kind of anti-diabetes drug, glucagon-like peptide-1 receptor agonist exenatide (exendin-4), on endometrial cancer. To investigate whether exenatide promotes or inhibits the growth of endometrial cancer, we used the subcutaneous human endometrial cancer cell Ishikawa xenografts in nude mouse model, and divided them into control group and exenatide-treated group. The tumor growth rate in exenatide group was slower than that in control group, and the apoptosis rate of exenatide group was higher than that in control group. *In vitro*, exendin-4 also attenuated Ishikawa cell viability and clone formation rate, but promoted cell apoptosis. There was an increase of phosphorylated-AMPK protein, a decrease of phosphorylated-mTOR protein both *in vivo* and *in vitro* after exenatide or exendin-4 treatment. Moreover, when treated with exendin-4 plus AICAR, an AMPK activator, cell apoptosis increased with higher ratio of phosphorylayed-AMPK/AMPK, lower ratio of phosphorylated-mTOR/mTOR and higher expression of cleaved caspase-3 than those in exendin-4 alone group, and the results were the opposite when treated with exendin-4 plus compound C, an AMPK inhibitor. Our results suggest that exenatide could attenuate the growth of endometrial cancer Ishikawa xenografts in nude mice, and AMPK may be the target of the mechanism.

Introduction

Endometrial cancer is a common gynecologic malignant tumor and the fourth malignant tumor for women in developed countries (1). A large number of studies showed that obesity, diabetes and insulin resistance are the high risk factors of endometrial cancer (2,3). The incidence of endometrial cancer in patients with diabetes was two times higher than that of non-diabetics (4). Diabetes also increased the mortality of endometrial cancer (5,6).

The risks for anti-diabetes drugs with malignant tumor are also of intensive concerned in recent years. Research shows that metformin could reduce the risk of certain cancers (7), and the mechanism was that metformin as an AMP-activated protein kinase (AMPK) agonist inhibits mTOR phosphorylation (8,9). Moreover, insulin may increase the risk of some cancers through insulin growth factor-1 receptor (IGF-1R)/PI3K/Akt/mTOR signaling pathway (10,11). The relationship between diabetes medication and tumor has attracted considerable attention. The question of whether the new anti-diabetes drug glucagon-like peptide-1 receptor (GLP-1R) agonist, such as exenatide (exendin-4), exerts some effects on the tumor is of interest to researchers.

Clinical trials showed that exenatide could significantly reduce fasting blood glucose and glycosylated hemoglobin in patients with diabetes, also promoted β cell proliferation and inhibited β cell apoptosis (12,13). Studies have shown that GLP-1 could inhibit the apoptosis of myocardial cells and neuronal cells, and its mechanism might be the inhibition of apoptotic pathways including PI3K-Akt-mTOR pathway and MAPK pathway (14,15). Based on the above, whether exenatide promotes tumorigenesis or tumor growth has also been concidered. Recent studies showed that exendin-4 did not enhance proliferation of pancreatic adenocarcinoma cells (16), but inhibited the growth of tumor cells of breast and colon cancer (17,18).

Previous studies revealed that exendin-4 could inhibit lipid synthesis of hepatic cells by upregulation of AMPK phosphorylation (19,20). In addition, AMPK has been confirmed as the key of a series of complex molecular events, far more than the cell energy metabolism in the body. AMPK is in the pivotal site of PI3K-AKT-mTOR and other signal pathways, and mTOR signaling pathway can promote apoptosis, which affects the occurrence and development of tumors. The above findings will bring new opportunities for cancer treatment (21).

Whether GLP-1R agonist exenatide also have effects is not known. Therefore, we designed experiments *in vivo* and *in vitro* to investigate the role of GLP-1R agonist on endometrial cancer.

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Materials and methods

Human tissues. Human normal endometrium tissues were obtained from 10 patients (42-48 years old) who received hysterectomy due to uterine leiomyoma without endometrial disease, and endometrial cancer tissues were obtained from 10 patients (45-55 years old) who received hysterectomy due to endometrial cancer (type 1) at the Third Affiliated Hospital of Sun Yat-sen University from January 2014 to July 2014. The tissues were paraffin-embedded, formalin-fixed and cut into 4 μm sections for immunohistochemistry staining. All the patients provided written informed consent for participation in the present study. The study protocol was approved by the Ethics Committees of the Third Affiliated Hospital of Sun Yat-sen University.

Animal studies. Female 4-week-old BALB/c mice were obtained from Beijing Laboratory Animal Research Center (Beijing, China) and maintained in specific pathogen free facilities approved by the Chinese Association for Accreditation of Laboratory Animal Care with an approved protocol by the Institutional Animal Care and Use Committee. Experiments were performed under institutional guidelines established for Biomedical Research Center, The Third Affiliated Hospital, Sun Yat-sen University. After 1 week of acclimation, Ishikawa cells were injected subcutaneously into the right flank of mice respectively ($6x10^6$ cells/mouse). One week later, the mice were randomly divided into two groups (5 mice per group), given intraperitoneal injection of exenatide (Eli Lilly and Company, Indianapolis, IN, USA) (24 nmol/kg/d) to the mice in exenatide group and physiological saline to control group, 6 day/week, for 4 weeks. Tumor size was measured with a linear digital caliper every 3-4 days. Tumor volume was estimated using the equation $V = (axb^2) \times 0.5236$, where 'a' is the larger imension and 'b' is the perpendicular diameter. Weights of BALB/c mice were measured using electronic balance every 3-4 days during the medical treatment. Observations of BALB/c mouse diet, activities and mental state in general were also recorded.

Endometrial cancer cell line. Human endometrial carcer cell line Ishikawa was provided by the American Type Culture Collection (ATCC; Manassas, VA, USA). The cells were routinely cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco, Carlsbad, CA, USA) supplied with 10% fetal calf serum (FBS; HyClone Laboratories, Inc., South Logan, UT, USA), at 37˚C in the humidified atmosphere of a 5% CO_2 incubation.

Random blood glucose. Before treatment and every 7 days during intervention, random blood glucose of the mice was monitored through the tail veins by superior blood glucose meter (Roche, Basel, Switzerland).

Harvesting samples. At the end of the experiment, the mice were fasted for 8 h, anesthetized and sacrificed for blood and tissue collection. Blood samples were collected through the periorbital venous of the mice and centrifuged for serum for the detection of GLP-1, insulin and IGF-1 value. In addition, the harvested tumors were weighed and separated for preservation in 4% neutral formaldehyde solution at -80˚C. Then, the tumor tissues preserved in formaldehyde solution were paraffin-embedded, formalin-fixed and cut into 4 μm sections for immunohistochemistry staining, and the samples preserved at -80˚C were used for western blot analysis.

Enzyme-linked immunosorbent assay (ELISA). ELISA was performed with the serum samples of the mice for the detection of GLP-1, insulin and IGF-1 value according to the manufacturer's instructions, respectively of Glucagon-like peptide-1, total ELISA kit (Merck Millipore, Darmstadt, Germany), insulin (Abcam, Cambridge, UK) and mouse/rat IGF-1 immunoassay (Merck Millipore).

Immunohistochemistry analysis and evaluatoin. The human and animal tissue slides were deparaffinized in xylene, rehydrated through graded alcohol, immersed in 3% hydrogen peroxide for 10 min to block endogenous peroxidase activity, and antigen retrieved by pressure cooking for 3 min in Tris/EDTA (pH 8.0). The slides were then incubated with the primary antibody of GLP-1R antibody (ab39072, 1:50 dilution; Abcam), rabbit anti-Ki-67 (1:100 dilution; Merck Millipore), DNA fragmentation detection kit, fluorescent-TdT enzyme (1:1,000 dilution; Merck Millipore) for 1 h at room temperature according to the manufacturer's instructions. After being incubated with the secondary antibody for 30 min, specimens were stained with DAB (3,3-diaminobenzidine). Finally, the sections were counterstained with hematoxylin, dehydrated and mounted. Slides were examined with a Leica microscope (Leica Microsystems GmbH, Wetzlar, Germany).

Cell viability assay. Cell viability were measured using a 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (MP Biomedicals LLC, Santa Ana, CA, USA). Ishikawa cells ($2-3x10^3$) were seeded in 96-well plates at varied density ($2-3x10^3$/well), cultured in the appropriate media and grown to 70% confluence. Exendin-4 (Sigma-Aldrich, St. Louis, MO, USA) was added to culture medium at the indicated concentrations for different times. Twenty microliters of MTT (5 mg/ml) was added to each well, and cells were subsequently incubated at 37˚C for an additional 4 h. Crystals were dissolved in 150 ml of DMSO. Absorbance was measured at a wavelength of 490 nm using a microplate reader (ELx800; Bio-Tek Instruments, Inc., Winooski, VT, USA).

Colony assays. Fifty cells/well were plated on 96-well plates. Twenty-four hours later, medium was replaced, and cells were incubated with exendin-4 at indicate concentration. Medium was replaced every day, and at day 14, the cells were fixed and stained using 0.5% crystal violet (Sigma-Aldrich).

Apoptosis analysis. Cells ($7x10^5$) were plated in the appropriate culture media containing 10% FCS in 12-well plates. Cells were then serum starved for 24 h, and treated with exendin-4, AICAR (Sigma-Aldrich) or compound C (Sigma-Aldrich) at indicated concentration. After 48 h, cells and medium were collected and stained with PI and Annexin V, using the Annexin V-PE apoptosis detection kit I (Becton-Dickinson, Franklin Lakes, NJ, USA) according to the

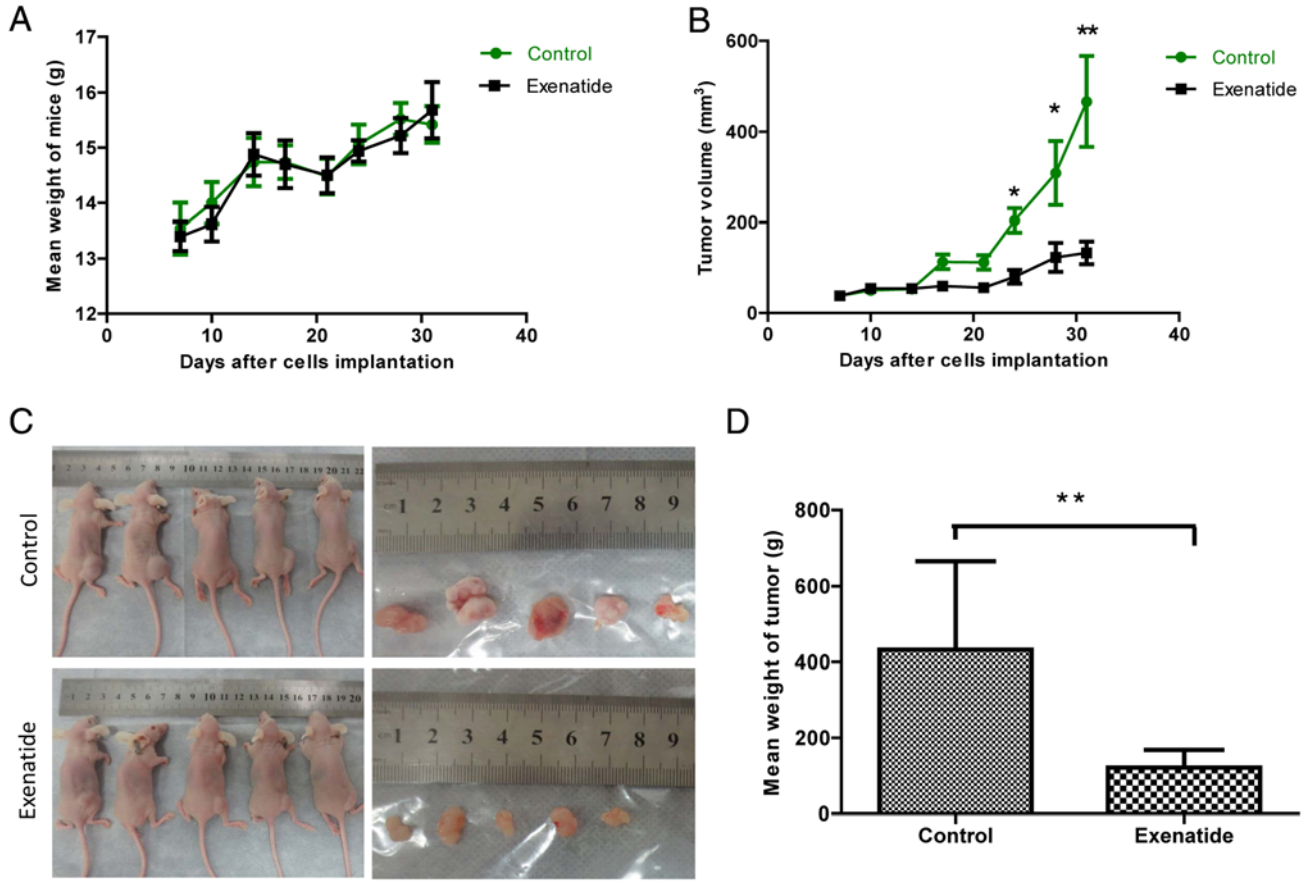

Figure 1. Exenatide inhibited the growth of Ishikawa xenografts in nude mouse model. (A) Data shown are the means ± SD from physiological saline or exenatide-treated mice (n=5 mice per group) (P>0.05). (B) Tumor volumes were measured during the intervention (*P<0.05; **P<0.01). (C) At the end of intervention, the nude mice with tumor (left panels), and the harvested tumors (right panels). (D) The mean weights of tumors at the end of intervention are shown (**P<0.01).

manufacturer's protocol. Then flow cytometry evaluation was performed using flow cytometry (Becton-Dickinson).

Western blot analysis. Tumor tissue and cells were harvested, lysed and total protein was extracted with RIPA buffer (50 mM Tris-Cl pH 7.4, 150 mM NaCl, 1% NP-40, 0.25% Na-deoxycholate, 1 mM EDTA, 1 mM NaF) together with a protease inhibitor cocktail (Sigma-Aldrich). Lysates were resolved on 10% SDS-PAGE and immunoblotted with the indicated antibodies as GLP-1R antibody (ab39072; Abcam), AMPKa antibody (Cell Signaling Technology, Beverly, MA, USA), Phospho-AMPKa (Thr172) antibody (Cell Signaling Technology), mTOR antibody (Cell Signaling Technology, Beverly), Phospho-mTOR (Ser2448) antibody (Cell Signaling Technology), caspase-3 (Cell Signaling Technology), GAPDH antibody (Cell Signaling Technology) and β-actin (Cell Signaling Technology). The membranes were incubated with secondary antibodies (1:10,000, DyLight 800; Thermo Fisher Scientific, Waltham, MA, USA) at room temperature for 1 h. The membranes were imaged with the Odyssey Infrared Imaging System (LI-COR Biosciences, Lincoln, NE, USA).

Statistical analysis. The data are expressed as mean ± SD. The study variables were compared between the study groups using t-test for normal distribution, grouped Wilcoxon rank sum test for skewed distribution. All the calculations were two tailed. P<0.05 was considered as statistically significant.

Results

Exenatide inhibits the growth of endometrial cancer Ishikawa xenografts in nude mice. At the 7th day after Ishikawa cell implantation, the mean tumor volume (V) of subcutaneous tumor in nude mice was 38.4±9.92 mm^3, and the mice were randomly divided into control group [N=5, V=(38.4±9.1) mm^3] and exenatide group [N=5, V=(38.4±11.7) mm^3]. During the intervention, diet, activities and mental state of the nude mice were normal, and all were alive until the end of the intervention. The mouse body weight of two groups increased stably during the experiment period without significant differences (P>0.05; Fig. 1A). According to the tumor growth curves, the tumor growth rate of exenatide group was slower than that in control group, especially at days 24, 28 and 31 (P<0.05, P<0.01; Fig. 1B). At the end of the intervention, the mean tumor volume of exenatide group and control group was 125.9±20.4 and 440.2±117.1 mm^3, respectively (P<0.01; Fig. 1B), and the mean tumor weight of exenatide group (116.9±47.1 mg) was significantly lower than that in control group (440.2±142.5 mg) (P<0.01; Fig. 1C and D).

Higher serum GLP-1 level in exenatide group. Random blood glucose of the mice was monitored every 7 days during the experiment, and it was in the normal range of 5-7.4 mmol/l in exenatide group and 4.9-7.7 mmol/l in control group, respectively (P>0.05; Fig. 2A). At the end of the intervention, serum

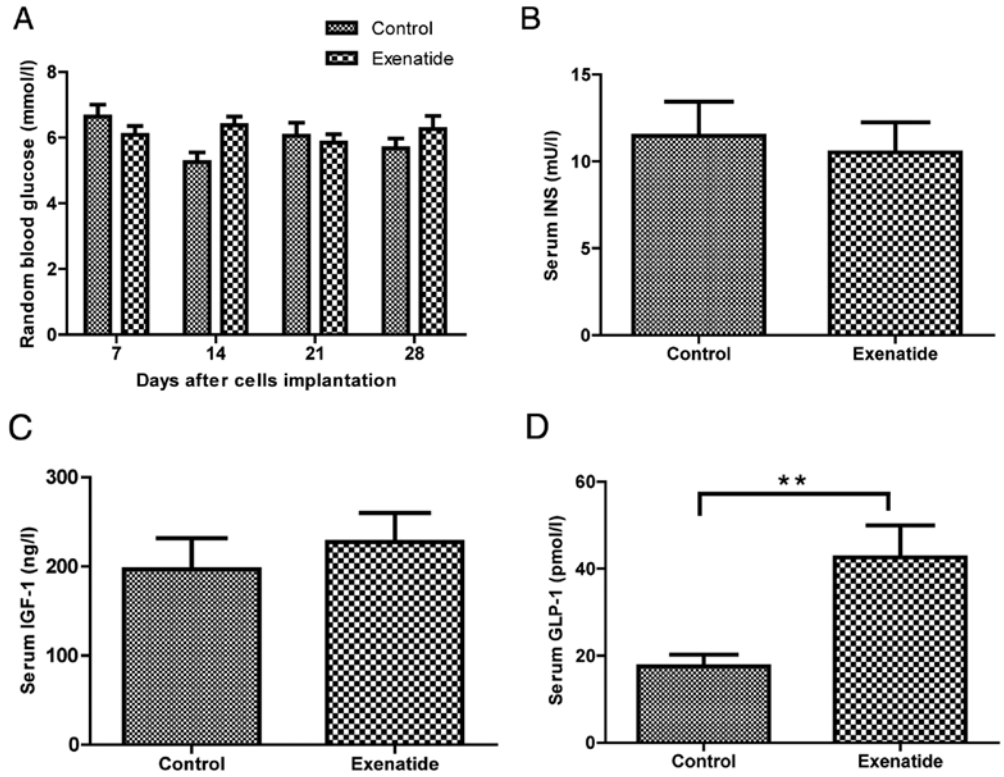

Figure 2. Exenatide did not change blood glucose, serum insulin and IGF-1 levels except for the GLP-1 level in the nude mice. (A) Random blood glucose ($P>0.05$). (B) Serum insulin value ($P>0.05$). (C) Serum IGF-1 value ($P>0.05$). (D) Serum GLP-1 value ($^{**}P<0.01$).

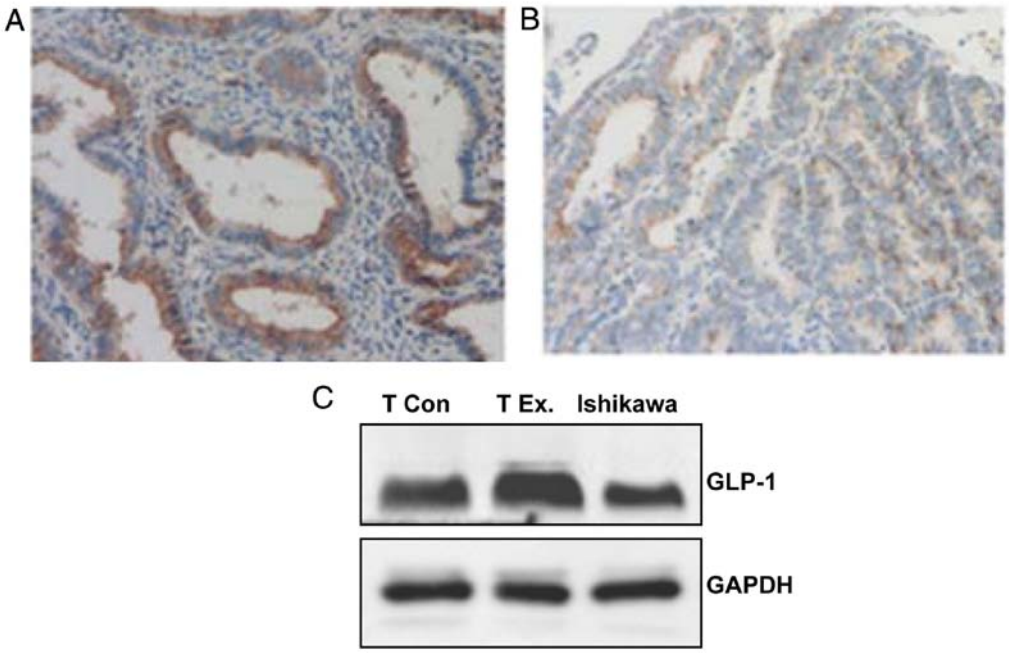

Figure 3. GLP-1 receptor expressed in endometrial cancer tissue and cells. (A and B) GLP-1R was detected by immunohistochemistry in human normal endometrium tissue (A) and endometrial cancer tissues (B). The brown granules in the cell membrane and cytoplasm are GLP-1Rs. Magnification, x200. (C) GLP-1R detection by western blot analysis in Ishikawa cells and the tumor tissue of control group (T con) and exenatide group (T Ex.) of Ishikawa cell xenografts in nude mice. GAPDH was used as internal control.

insulin value was 11.09±1.60 mU/l in exenatide group and 9.05±0.85 mU/l in control group without significant difference ($P>0.05$; Fig. 2B). Serum IGF-1 value was 265.6±8.4 ng/ml in exenatide group and 276±8.1 ng/ml in control group without significant difference ($P>0.05$; Fig. 2C). However, serum GLP-1 value of the nude mice was 41.01±4.78 pmol/l in exenatide group and 15.70±0.73 pmol/l in control group with significant difference ($P<0.01$; Fig. 2D). Thus, exenatide did

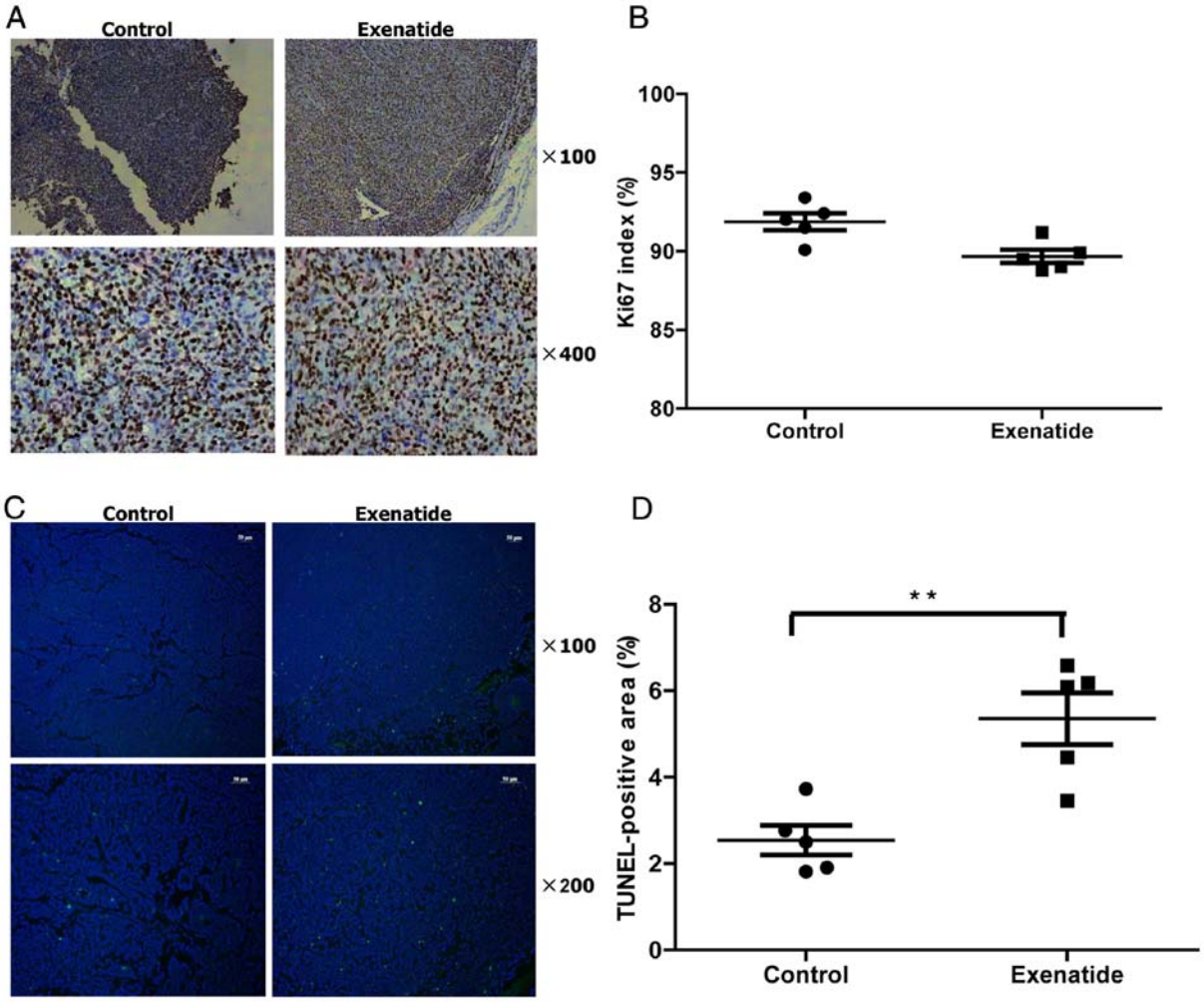

Figure 4. Exenatide promotes apoptosis. (A) Ki-67 positive cell nucleus has brown granules. Data shown are tumor cell proliferation index of each individual tumor with the mean indicated by a horizontal line ± SE (P>0.05). (B) Green fluorescence within the nucleus shows apoptotic bodies. Data shown are TUNEL-positive area (as a percentage of the entire tumor area) of each individual tumor with the mean indicated by a horizontal line ± SE (**P<0.01).

not change blood glucose, serum insulin or IGF-1 level in the non-diabetes mice. In addition, higher serum GLP-1 level in exenatide group was observed.

GLP-1 receptor is expressed in endometrial cancer tissue and cells. We attempted to detect GLP-1R in the endometrial cancer tissue and cells. Immunohistochemical analysis of GLP-1R was performed in human normal endometrium tissues (Fig. 3A) and endometrial cancer tissues (Fig. 3B). GLP-1R was expressed in all of the 20 cases. GLP-1R was also detected by western blot analysis in Ishikawa cell line and the tumor tissue of the Ishikawa cell xenografts in nude mice, and the results were positive (Fig. 3C). Thus, higher level of GLP-1 might bind to GLP-1R to make function directly.

Exenatide promotes apoptosis to attenuate tumor growth. The proportion of Ki-67 positive cells of the entire tumor cells stands for the tumor proliferation index, and it was (89.6±0.9%) in exenatide group and (91.1±1.35%) in control group, respectively, without significant difference (P>0.05; Fig. 4A). Immunofluorescence TUNEL detection showed that the apoptosis rate was significantly higher in exenatide group (5.4±1.3%) than that in control group (2.5±0.7%) (P<0.01; Fig. 4B). Effect of exendin-4 on endometrial cancer cell line Ishikawa growth was determined by MTT assay and colony formation assay. The results showed that exendin-4 did attenuate the viability of Ishikawa cells (P<0.05, P<0.01; Fig. 5A and B). Based on the result of the MTT assay, apoptosis rate was detected when Ishikawa cells were treated with 0 or 10 nM of exendin-4 for 48 h, and the apoptosis rate was significantly higher in the group treated with 10 nM of exendin-4 (P<0.01; Fig. 5C and D).

Exenatide regulates AMPK-mTOR signaling to promote apoptosis. The role of AMPK has been proved as the key of a series of complex molecular events, and AMPK-mTOR signaling pathway regulates apoptosis (21). To test whether the apoptosis action of exenatide is mediated by AMPK-mTOR signaling, protein level of AMPK and mTOR were examined in Ishikawa xenografts. The results showed that phosphorylated-AMPK protein increased and phosphorylated-mTOR protein decreased (Fig. 6A). As confirmation, we examined AMPK-mTOR signaling *in vitro*. Again, we observed that phosphorylated-AMPK protein increased, phosphorylated-mTOR protein decreased and cleaved caspase-3 increased in exendin-4 group (10 nM) (Fig. 6B). Moreover, when

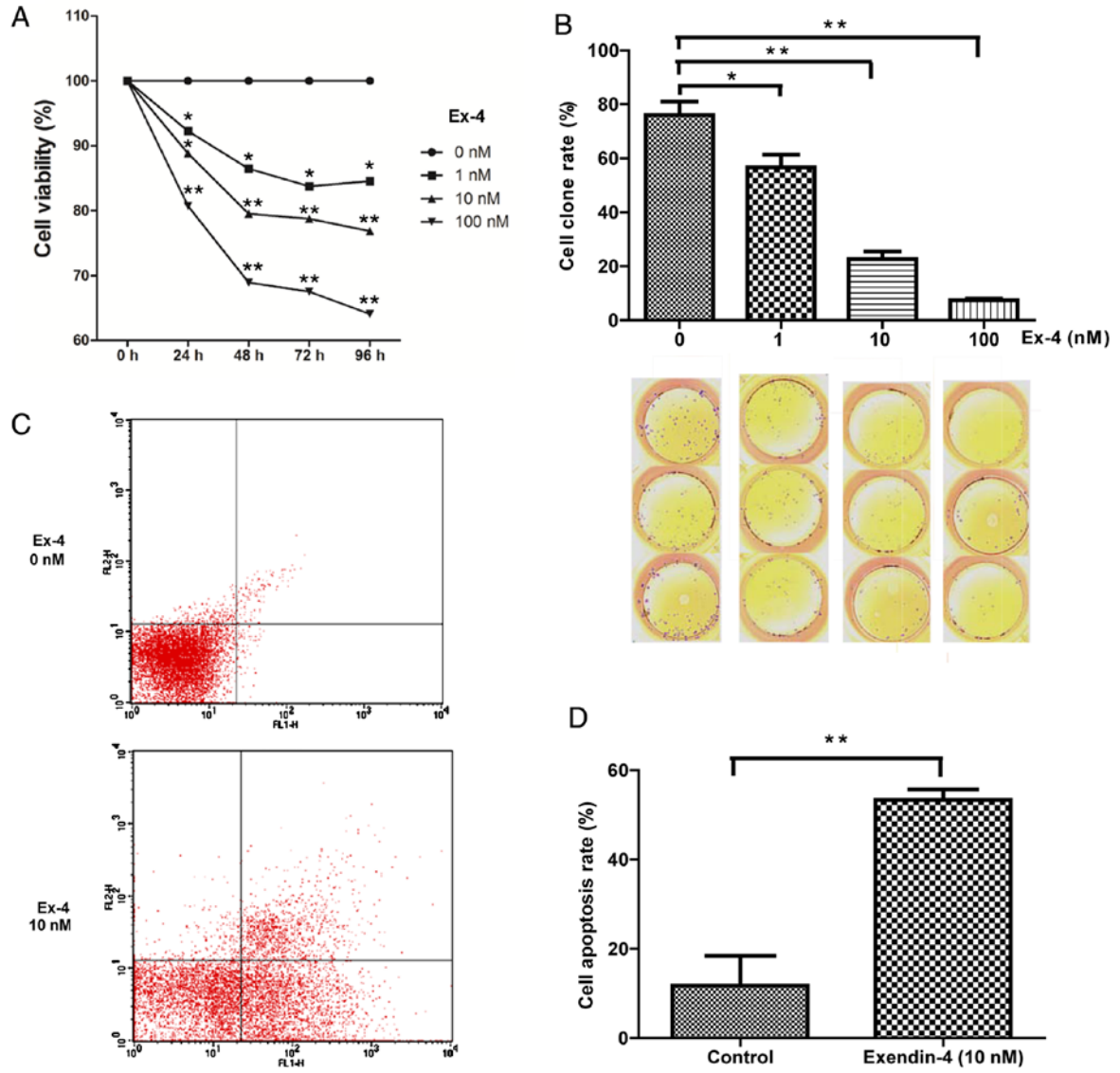

Figure 5. Exendin-4 inhibits endometrial cancer Ishikawa cell line viability. (A) MTT assay result, comparing to 0 nM, Ishikawa cell viability with 1, 10 and 100 nM of exendin-4 (Ex-4) were significantly lower at 24, 48, 72 and 96 h (*P<0.05, **P<0.01). (B) Ishikawa cells were treated with Ex-4 (0, 1, 10 and 100 nM). The whole pictures of clones are shown with bar graphs (*P<0.05, **P<0.01). (C and D) Apoptosis analysis, Ishikawa cells were treated with Ex-4 (0 or 10 nM) for 48 h, harvested and stained as described in Materials and methods. Representative results are shown (C). Results of three independent experiments are shown in bars (D) (**P<0.01).

Ishikawa cells were treated with exendin-4 plus AICAR, an AMPK activator, cell apoptosis increased with higher ratio of phosphorylated-AMPK/AMPK, lower ratio of phosphorylated-mTOR/mTOR and higher expression of cleaved caspase-3 than those in exendin-4 group, and the results were the opposite when cells were treated with exendin-4 plus compound C, and AMPK inhibitor (Fig. 7). Thus, exendin-4 might phosphorylate AMPK which results in the reduced phosphorylation of mTOR and promoted apoptosis.

Discussion

Exenatide, as an early listed GLP-1 receptor agonist, has become a widely-used anti-diabetic treatment throughout the world with many benefits. However, the current considerable interest in incretin therapy has raised the issue of its long-term safety including the risk of carcinogenesis. We report in the present study that exenatide could attenuate endometrial cancer Ishikawa cell xenografts growth, which is consistent with the results of the studies reported recently. Chen *et al* (22) reported that exendin-4 enhances the effect of chemotherapy in bile duct carcinoma *in vivo*. Another study (23) revealed that a GLP-1 analogue liraglutide inhibits the growth of pancreatic cancer in an animal model. In addition, Honors *et al* (24) found that the application of exendin-4 in animal models of malignant ascites tumor inhibits the tumor growth and improve cachexia, and Nomiyama *et al* (25) showed exendin-4 attenuates prostate cancer growth.

Obesity and T2DM have been found to be associated with increased endometrial cancer risk and adverse prognosis among endometrial cancer patients (2-6). The mechanisms involved in this interaction are not fully elucidated and might be related with the increased blood glucose, serum insulin levels, activation of the IGF-1 pathway, and production of

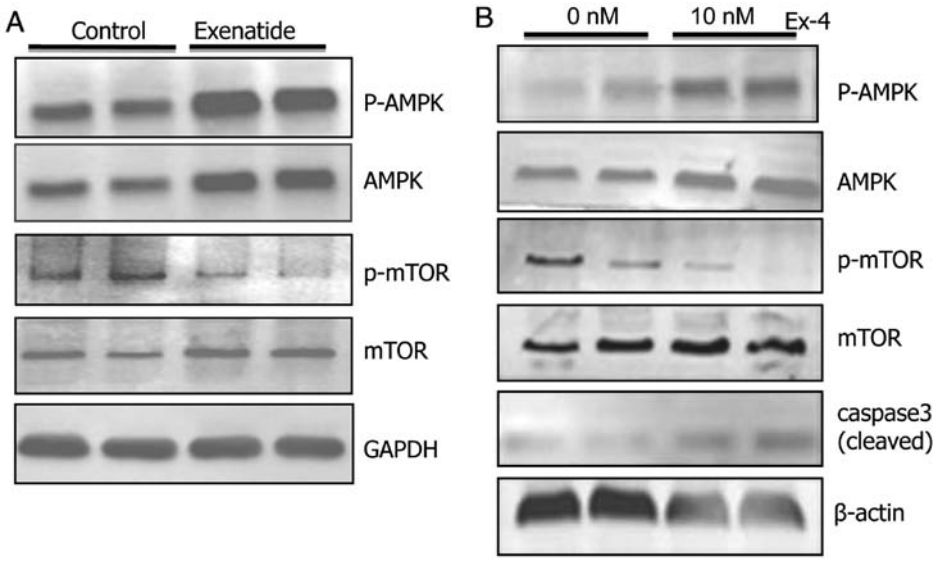

Figure 6. Exenatide regulates AMPK-mTOR signaling to promote apoptosis. (A) Total protein extracted from Ishikawa xenograft lysates was used in western blot analysis. AMPK, p-AMPK, mTOR and p-mTOR were detected with specific antibodies. GAPDH was used as an internal control. (B) Ishikawa cells were treated with 0 and 10 nM of Ex-4 for 48 h, harvested and analyzed by western blot analysis for the expression of AMPK, p-AMPK, mTOR, p-mTOR and caspase-3 (cleaved), β-actin was used as internal control.

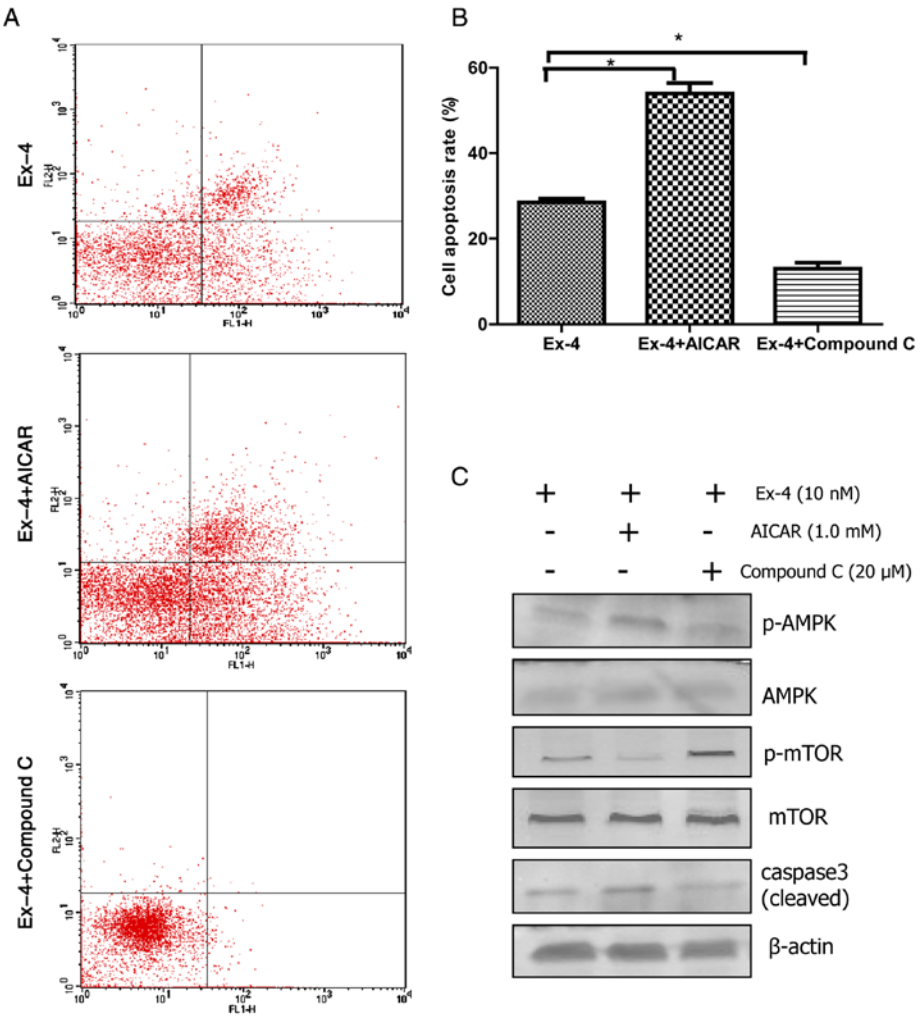

Figure 7. AMPK activator enhances exendin-4 action on Ishikawa cells. (A and B) Apoptosis analysis, Ishikawa cells were treated with Ex-4 (10 nM), Ex-4 (10 nM) + AICAR (1.0 mM), Ex-4 (10 nM) + compound C (20 μM) for 48 h, harvested and stained as described under Materials and methods. Representative results are shown. (A). Results of three independent experiments are shown in bars (B) (*P<0.05). (C) Ishikawa cells were treated with Ex-4 (10 nM), Ex-4 (10 nM) + AICAR (1.0 mM), Ex-4 (10 nM) + compound C (20 μM) for 48 h and harvested for analyzing the expression of AMPK, p-AMPK, mTOR, p-mTOR and cleaved caspase-3 by western blot analysis. β-actin was used as an internal control.

sex hormones by the adipose tissues (26). In this study, we found that exenatide elevated the serum GLP-1 level but had no significant effect on blood glucose, insulin and IGF-1 levels (Fig. 2) in the healthy young nude mice. The higher serum GLP-1 in exenatide-treated group might be one of the factors of inhibiting the human endometrial cancer Ishikawa cell xenograft in nude mice. We detected GLP-1R in human endometrial cancer cells, and the results suggested that GLP-1R is abundantly expressed both in cancerous and non-cancerous endometrial cells (Fig. 3), which is consistent with the results of classic or functional GLP-1R existing in breast cancer, colon cancer and prostate cancer cells (17,18,25). Thus, exenatide-attenuated endometrial cancer growth might be mediated by GLP-1R signaling.

Endometrial cancer is strongly associated with obesity and diabetes (2,3). The IGF system has also been linked with obesity, diabetes, hyperinsulinemia, and several human malignancies including endometrial cancer (27). The signal pathway of IGF-1R/PI3K/Akt/mTOR had been revealed to be upregulated in endometrial cancer in our previous study and other studies (28-30). Moreover, mTOR is upregulated in many cancers as a result of genetic alterations or aberrant activation of components of the PI3K/AKT pathway, which contributes to the dysregulation of cell proliferation, growth, differentiation and survival (31-33). The PI3K/AKT/mTOR pathway was inhibited by phospholipids and phosphatases, such as PTEN, and resulted in tumor suppression (34,35). AMPK plays an important role among a series of complex molecular events, including lipid metabolism, carbohydrate metabolism, protein synthesis, cell apoptosis, angiogenesis, anti-inflammatory, and regulation on cell senescence (36,37). AMPK is at the pivotal site of PI3K-AKT, mTOR and other signal pathways (21). AMPK activation triggers the regulation of multiple downstream pathways, including mTOR. AMPK mediates its effect on cell growth through inhibition of mTOR (38), and mTOR signaling pathway promoted the apoptosis-related protein caspase-3 expression to affect the occurrence and development of tumor, which has brought new opportunities for the treatment of metabolic diseases and cancer (21). In our *in vivo* study, the mechanism of exenatide inhibiting the growth of endometrial cancer cell Ishikawa xenografts, maybe at least partly includes activating AMPK phosphorylation and results in inhibiting mTOR phosphorylation to promote apoptosis (Fig. 6A).

Further verification was conducted *in vitro*. From the results of MTT and cloning analysis with exendin-4 of different concentrations, exendin-4 inhibited the growth of endometrial cancer Ishikawa cells at a dose- and time-dependent manner (Fig. 5A and B). Exendin-4 promoted Ishikawa cell apoptosis (Fig. 5C and D), and the expression ratios of phosphorylated-AMPK/AMPK and phosphorylated-mTOR/mTOR were consistent with the result *in vivo*, which resulted in increasing the expression level of apoptosis protein caspase-3 (Fig. 6B).

The *in vitro* study (17) on breast cancer cells suggests that exendin-4 can increase intracellular cyclic adenosine monophosphate (cAMP) value which promotes cAMP related apoptosis factor p38 expression through functional GLP-1R. The study (18) on colon cancer cells shows that exendin-4 also increases the expression of apoptosis protein caspase-3 by GLP-1R signaling. The study (25) on prostate cancer suggested that exendin-4 attenuates prostate cancer growth through GLP-1R signaling of inhibition of ERK-MAPK activation. Exendin-4 was able to upregulate AMPK phosphorylation to inhibit lipid synthesis (19,20), reduce glomerular mesangial cell proliferation and fibronectin in high glucose induced rats partly through upregulation of AMPK phosphorylation (39). Thus, we preliminarily conclude that exenatide (exendin-4) inhibits endometrial cancer Ishikawa growth through phosphorylating AMPK through GLP-1R signaling, which is similar to the mechanism of metformin on endometrial cancer cells (7-9). We conducted an experiment to confirm the results showing AMPK agonist AICAR enhanced Ishikawa cell apoptosis by upregulating AMPK phosphorylation, while the AMPK inhibitor compound C effect was the opposite (Fig. 7).

The present study suggested that exenatide did not enhance endometrial cancer growth, but even promote apoptosis to slow down the growth of endometrial cancer both *in vivo* and *in vitro*. However, studies on this class of medications on malignant diseases is limited. More endometrial carcer cell xenograft models, with different dose groups and positive control group with diabetes need to be conducted to confirm the results conclusively. The mechanistic and epidemiological investigations on whether this class of medication affects the biological behavior or risk of endometrial cancer, or other malignant disease development are important.

In conclusion, our results suggest that exenatide could attenuate the growth of endometrial cancer Ishikawa xenografts in nude mice, and AMPK may be the target of the underlying mechanism.

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