Liraglutide suppresses the proliferation of endometrial cancer cells through the adenosine 5′-monophosphate (AMP)-activated protein kinase signaling pathway

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This study investigated the effect of liraglutide (an analog of human glucagon-like peptide-1 [GLP-1]) on the proliferation of Ishikawa (IK) and human endometrial carcinoma (HEC-1B) endometrial cancer (EC) cell lines, identified whether liraglutide enhances the anti-tumor effect of progesterone, and explored the role of liraglutide in progesterone receptor (PGR) expression in EC cell lines and potential pathways.

IK and HEC-1B cells were plated into 96-well plates at densities of 8 × 10^4 and 1 × 10^5 cells/well, respectively. Twenty-four hours after being plated, the cells were serum-starved for an additional 24 h and were then treated with increasing concentrations of liraglutide (0.1, 0.5, 1, 2, 5, and 10 µmol/L; 0 µmol/L served as the blank control) for 24 h. About 10 µL of cell counting kit-8 (Dojindo, Shanghai, China) was added to each well. The absorbance of the samples was measured in an enzyme-linked immunosorbent assay reader at 450 nm. The serum-starved cells were treated with increasing concentrations of liraglutide (1, 2, and 5 µmol/L) in the absence or presence of increasing concentrations of medroxyprogesterone acetate (MPA; 0, 5, and 10 mmol/L) for 48 h; subsequently, the optical density values were measured. The effect of the combined application of liraglutide and progesterone on cell proliferation was assessed by calculating the combination index (CI) values using Compusyn Software (ComboSyn, Paramus, NJ, USA). The CI, which is derived from the median-effect principle of Chou and Talalay, provides a quantitative measure of the degree of interaction between two or more agents. CI values of over 1.1, 0.9 to 1.1, 0.7 to 0.9, 0.3 to 0.7, and <0.3 indicate antagonistic, additive, moderately synergistic, synergistic, and strongly synergistic effects, respectively. For real-time reverse transcription-polymerase chain reaction, IK and HEC-1B cells were plated at 2 × 10^5 cells/well into six-well plates for 24 h and were then treated with liraglutide (1, 2, and 5 µmol/L) in the presence or absence of Compound C. The following primer sequences were used: PGR (f): 5′-CATGATGCTGTATTTGCACTGAT-3′, PGR (r): 5′-CTCCTGGCTTACCTGAGCTTG-3′, glyceraldehyde-3-phosphate dehydrogenase (GAPDH, f): 5′-CAGTCAGCCGATCTTCTTTT-3′, and GAPDH (r): 5′-GTGACCAGGGCCCAATAC-3′. The obtained messenger ribonucleic acid (mRNA) levels of PGR were acquired by normalizing the threshold cycle (Ct) of PGR to the Ct of GAPDH. For Western blotting, IK and HEC-1B cells were plated at 2 × 10^5 cells/well into six-well plates for 24 h and were then treated with liraglutide (1, 2, and 5 µmol/L) in the presence or absence of Compound C to observe the changes in PGR, p-adenosine 5′-monophosphate (AMP)-activated protein kinase (p-AMPK), and p-70-kDa ribosomal protein S6 kinase (p-P70S6K) protein levels. The data were analyzed using an independent t-test and factor analysis of variance by applying Statistical Package for the Social Sciences (SPSS, version 20.0, Chicago, IL, USA). A P value of <0.05 was considered significant.

In this study, liraglutide significantly suppressed the proliferation of EC cells in a time- and concentration-dependent manner. Higher concentrations of liraglutide exerted higher antiproliferative effects on EC cells. The liraglutide concentration of 0.5 µmol/L exerted significant antiproliferative effects on IK cells. Similarly, the liraglutide concentration of 1 µmol/L significantly inhibited the proliferation of HEC-1B cells. A longer treatment time with liraglutide exerted more significant growth inhibition effects. The inhibition effect of liraglutide on IK and HEC-1B cells was more significant when concentrations of 5 and 10 µmol/L, respectively, were used (P < 0.05). As time progressed, the proliferation of IK cells declined after...
Figure 1: (A) Effect of liraglutide alone and combined with MPA on IK and HEC-1B cells (CI %). Compared with the application of liraglutide alone, the antiproliferative effect on EC cells was more significant when liraglutide was combined with MPA. (B) Effect of liraglutide alone and combined with Compound C on mRNA levels of PGR in IK and HEC-1B cells. The liraglutide up-regulated mRNA levels of PGR in IK and HEC-1B cells. Compound C could inhibit these effects. (C) Effect of liraglutide alone and combined with Compound C on protein levels of PGR, p-AMPK, p-P70S6K in IK and HEC-1B cells. Liraglutide significantly up-regulated protein levels of PGR and p-AMPK and down-regulated protein levels of p-P70S6K. Compound C could inhibit these effects. *P < 0.05. CI%: Cell inhibition%; EC: Endometrial cancer; MPA: Medroxyprogesterone acetate; mRNA: Messenger ribonucleic acid; p-AMPK: p-Adenosine 5’-monophosphate (AMP)-activated protein kinase; PGR: Progesterone receptor; p-P70S6K: p-70-kDa ribosomal protein S6 kinase.
Liraglutide combined with MPA had synergistic antiproliferative effects (CI < 1).Treatment with liraglutide and MPA alone could inhibit the proliferation of EC cells (P < 0.05). Compared with the application of liraglutide alone, the antiproliferative effect on EC cells was more significant when liraglutide was combined with MPA [Figure 1A]. These two drugs had synergistic effects on EC cells.

Compound C partly attenuated up-regulated mRNA levels of PGR in EC cells induced by liraglutide. IK and HEC-1B cells were stimulated with 1, 2, and 5 μmol/L liraglutide for 48 h. mRNA levels of PGR were significantly higher than those in the control group (0 μmol/L; P < 0.05). The liraglutide concentrations of 1 and 2 μmol/L caused the highest up-regulation of mRNA levels of PGR in IK and HEC-1B cells (P < 0.05 for both). When liraglutide was combined with the AMPK pathway inhibitor Compound C, the mRNA levels of PGR in IK and HEC-1B cells decreased [Figure 1B]. IK and HEC-1B cells were stimulated with 1, 2, and 5 μmol/L liraglutide for 48 h. The protein levels of PGR were significantly higher than those in the control group (0 μmol/L; P < 0.05). Compound C could inhibit these effects [Figure 1C]. Similar to PGR protein levels, the protein levels of p-AMPK were significantly higher than those in the control group. And for P70S6K (a downstream effector of AMPK), liraglutide could down-regulate the protein levels of p-P70S6K. Compound C could inhibit the effect [Figure 1C].

Progesterone is a classical, safe, and effective hormone therapy for young, IA stage, non-muscular infiltration, highly differentiated, PGR-positive EC patients. However, progesterone resistance is common in clinical practice, with a prevalence of approximately 25%. Therefore, identifying new agents to up-regulate the PGR expression will improve the effectiveness of hormone therapy with progesterone. Much research attention has been devoted to metabolic syndrome (MS), especially obesity and type 2 diabetes mellitus (T2DM), which is a risk factor for EC. Applying effective agents against MS reduces the incidence of EC and improves its prognosis. The newly developed diabetes drug liraglutide, which is a GLP-1 analog, provides a new treatment choice for MS. Currently, it is widely used in the treatment of obesity and T2DM; however, its application for cancer treatment is rare, and its molecular mechanism is unclear.

The GLP-1 analog exenatide remarkably decreased endometrial injury and fibrosis in rats. These effects of exenatide may prevent endometrial disorders in women with diabetes. Recently, Zhang et al. found that exenatide inhibited the proliferation of IK cells, and targeting the AMPK pathway may be the underlying mechanism. A systemic review including eight trials involving 5531 patients demonstrated that exenatide or exenatide long-acting release was inferior to liraglutide in controlling glycemia and facilitating weight loss. Based on these findings, we sought to determine whether liraglutide also inhibits the proliferation of EC cells and whether it increases the PGR expression and alleviates the progesterone resistance in EC cells. In our study, we found that liraglutide could significantly suppress the proliferation of EC cells in a time- and concentration-dependent manner in IK and HEC-1B EC cells, which was in accordance with a previous study. We reported that liraglutide increased the expression of PGR and synergistically suppressed the proliferation of EC cells when combined with MPA. This indicated that liraglutide enhanced the progesterone sensitivity, which rendered the combined application of liraglutide and MPA, a possible treatment for precancerous lesions and EC in the future.

AMPK is a well-known serine/threonine-protein kinase that functions as an important modulator of energy homeostasis. Our data showed that the AMPK inhibitor Compound C could inhibit the up-regulation of PGR. These results suggest that AMPK acts as a vital sensor that regulates the PGR expression. Moreover, liraglutide could improve the p-AMPK expression and down-regulate the p-P70S6K, a downstream protein in the AMPK pathway. Evidence supports that AMPK activation inhibits cell proliferation by inactivating the downstream pathway. This finding suggests that liraglutide regulates both the upstream signal of the pathway (high p-AMPK expression) and downstream signal of the pathway (low p-P70S6K expression), which may decrease the energy consumption and ensure the viability of EC cells. Therefore, these signals are the potential targets for cancer treatment.

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Conflicts of interest
None

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