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Incretins in tumor–endothelial interaction

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Exenatide modulates tumor–endothelial cell interactions in human ovarian cancer cells

Endocrine CONNECTIONS

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Abstract

Diabetes and cancer are prevalent diseases whose incidence is increasing globally. Diabetic women have a moderate risk increase in ovarian cancer, suggested to be due to an interaction between these two disorders. Furthermore, patients manifesting both diseases have associated worse prognosis, reduced survival and shorter relapse-free survival. According to current recommendations, incretin drugs such as Exenatide, a synthetic analog of Exendin-4, and Liraglutide are used as therapy for the type 2 diabetes (T2D). We studied the effects of GLP-1 and Exendin-4 on migration, apoptosis and metalloproteinase production in two human ovarian cancer cells (SKOV-3 and CAOV-3). Exendin-4 inhibited migration and promoted apoptosis through caspase 3/7 activation. Exendin-4 also modulated the expression of key metalloproteinases (MMP-2 and MMP-9) and their inhibitors (TIMP-1 and TIMP-2). Vascular endothelial cells, which contribute to the formation and progression of metastasis, were also analyzed. TNF-α-stimulated endothelial cells from iliac artery after Exendin-4 treatment showed reduced production of adhesion molecules (ICAM-1 and VCAM-1). Additionally, incretin treatment inhibited activation of apoptosis in TNF-α-stimulated endothelial cells. In the same experiment, MMPs (MMP-1 and MMP-9), which are relevant for tumor development, were also reduced. Our study demonstrated that incretin drugs may reduce cancer cell proliferation and dissemination potential, hence limiting the risk of metastasis in epithelial ovarian cancer.

Background

Cancer and diabetes are growing health problems worldwide. Epidemiological data show an increase in the prevalence of those diseases. Their coexistence is more common than estimated on the basis of their frequency in the population suggesting a cause–effect interaction between cancer and type 2 diabetes (T2D) (1, 2). Furthermore, the coexistence of both diseases is associated with worse prognosis and higher mortality (3). In the clinical setting, several groups of drugs are used in the treatment of diabetes including insulin, sulfonylurea and metformin. Insulin and sulfonylurea treatment in patients under anti-cancer therapy have been associated with worse prognosis, lower overall survival rate and shorter time-to-relapse as compared to patients on metformin or

patients without diabetes. To date, metformin has proven to give beneficial effects in patients with cancer, leading to longer survival rates, better prognosis and *in vitro* inducing apoptosis of some tumor cell lines (4).

Alternatively, incretin mimetic drugs are a relatively new group of drugs used in the treatment of diabetes that are currently recommended by American Diabetes Association in dual therapy with metformin for the treatment of T2D (5, 6). The mechanism of action of incretin mimetic drugs is through the binding to glucagon-like peptide-1 receptor (GLP-1R) in pancreatic beta cells stimulating insulin secretion. The two most important natural incretin hormones are glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP). However, GIP and GLP-1 are not used as drugs, due to their rapid hydrolysis by dipeptidyl peptidase-IV (DPP-IV). Consequently, pharmacological approaches are focused on the use of GLP-1 analogs, such as Liraglutide and Exenatide (a synthetic version of Exendin-4), with extended half-life and resistance to DDP-IV enzymatic degradation. Interestingly, GLP-1R is present on various types of cells, among which include cancer cells (7, 8).

The high mortality rate in ovarian cancer patients is mostly due to a late diagnosis, at which time the cancer has metastasized throughout the peritoneal cavity and omentum (9). Epithelial ovarian cancer can disseminate via transcoelmic, hematogenous or lymphatic route (10). Cancer metastasis is facilitated by the remodeling of the extracellular matrix (ECM) at the tumor site (11) and during invasion of tissues (12). Breakdown of the ECM components is carried out by matrix metalloproteinases (MMPs), a family of proteolytic enzymes. MMP activity is tightly controlled mainly by tissue inhibitors of metalloproteinase (TIMPs). Ovarian cancer cells alter MMP/TIMP ratio creating a microenvironment promoting cancer cell migration and metastasis (11, 13). Interestingly, pro-inflammatory environment modulates ovarian cancer cells (9) and endothelial cells (14, 15) to stimulate the synthesis or activation of various MMPs to aid in tumor growth, invasion and eventual metastasis. Furthermore, increase in MMP-2 and MMP-9 production has been associated with increased angiogenic response by VEGF expression potentially affecting metastatic potential of cancer cells (16).

The role of GLP-1 analogs on cancer cell growth and invasion both *in vitro* and *in vivo* is yet to be elucidated. Incretins have shown to inhibit growth and enhance apoptosis of cancer cells through inhibition of the PI3K/Akt pathway for some cell lines of breast (17), colon cancer (18) and ovarian cancer (19). Thus, understating of the role of GLP-1 analogs has important clinical implication in the design of novel anti-cancer therapies emphasizing the potential benefits of combining both incretins with chemotherapy-cytostatic drugs. In the present study, we investigated whether incretin agonist, Exendin-4, influenced ovarian cancer and vascular endothelium and this had a carry-on effect on tissue remodeling.

Materials and methods

Cell lines

Human ovarian cancer cell lines, SKOV-3 and CAOV-3 (ATCC), were cultured in DMEM (Sigma) or McCoy (Sigma) medium, respectively, with 1% antibiotics (Sigma) and 10% FBS (Sigma) at 37°C in 5% CO_2 humidified incubator. Human Iliac artery endothelial cells and human aortic endothelial cells (Lonza, Basel, Switzerland) were cultured in EBM-2 culture medium as previously described (20). Three ovarian tumor cell lines were isolated *de novo* from anonymous patients undergoing surgical removal of the ovaries due to ovarian cancer with patients' consent. Biopsies were obtained after approval of the ethic committee of the Medical University of Silesia (KNW/0022/KB1/122/14). Prior to incubation, the ovarian cancer biopsies were washed in HEPES buffer to remove blood. Then, the sections were cut into pieces with a diameter of approx. 1 mm and placed in a culture Petri dish. Tumor cells were isolated using the 'explant method' (21, 22) and cultured under the same conditions as SKOV-3 cells.

Transwell migration assays

Ovarian cancer cell migration was conducted using an 8-μm transwell system (Greiner Bio-One, Kremsmünster, Austria) in a 24-well plate format. For migration assay, 2×10^5 cells were placed in the upper chamber of the transwell in 200 μL medium (0.5% FBS), Exendin-4 (50 nM, Sigma), GLP-1 (100 nM, Sigma), GLP-1 antagonist 9–36 (50 nM) (Tocris, Bristol, UK). Cells were pre-incubated with Exendin-4 for 24 h when appropriate. Cells were induced to actively migrate through membrane into the lower compartment containing 600 μL of DMEM and monocyte chemoattractant protein (MCP-1) at 10 nM (23). Cells were cultured for 24 h. Migrated cells in the underside of the membrane were detached with trypsin and fluorescently labeled with Calcein-AM (8 μM) for 45 min.

A number of migrating cells were assessed by measuring fluorescence (excitation wavelength of 485 nm and an emission wavelength of 520 nm) with a microplate reader on Infinite M200 (TECAN, Männedorf, Switzerland).

Viability, cytotoxicity and apoptosis assays

Ovarian cancer cells were seeded into a 96-well plate at a total density of 2×10^4 cells per well in appropriate cell culture medium. Cells were pre-incubated with Exendin-4 for 24 h when appropriate and then incubated with Camptothecin (100 nM) or Exendin-4 (50 nM) for 24 h. Viability, cytotoxicity and caspase activation were determined using ApoTox-Glo Triplex assay (Promega) according to the manufacturer's protocol.

Immunoblotting

Cells were lysed in PathScan lysis buffer (Cell Signaling Technology) containing phenylmethylsulfonyl fluoride (Sigma) and protease inhibitor (Roche). The protein samples were separated by SDS-PAGE. Proteins were transferred into low autofluorescence Immobilon-FL PVDF membrane (Millipore) and incubated with either antibody, GLP-1R (ab39072, Abcam) at 1:1000 or GAPDH (Sigma) at 1:5000. Goat anti-rabbit secondary antibody conjugated with fluorescent dye IRDye800 (LI-COR Biosciences, Lincoln, NE, USA) was used. The proteins of interest were visualized by using LICOR Odyssey Infrared Imaging System.

Fluorescent bead-based Luminex cytokine assay

Analysis of MMPs and TIMPs protein concentration was performed in cell medium using multiplex, bead-based (Luminex) assays on a Bio-Plex200 suspension array system according to each manufacturer's instructions. Data were acquired on a validated and calibrated Bio-Plex 200 system (Bio-Rad Laboratories) and analyzed with Bio-Plex Manager 6.0 software (Bio-Rad Laboratories) with a detection target of 50 beads per region, low RP1 target for CAL2 calibration and recommended doublet discriminator gates of 5000–25,000 for Bio-Plex. The median fluorescence intensity was measured. Bio-Plex Pro Human MMP 9-Plex Panel (Bio-Rad Laboratories) was used for detection of MMPs. Bio-Plex Pro Human Cytokine 27-plex assay (Bio-Rad Laboratories) was used for detection of TNF-α and VEGF. Analysis of phosphoIκB (Ser32) and IκB was performed in cell medium using multiplex, bead-based (Luminex xMAP, Merck) as

described earlier and according to manufacturer's instructions.

Determination of adhesion molecule levels

ICAM-1 and VCAM-1 protein concentrations in IAEC cells were quantified by Enzyme Linked ImmunoSorbent Assay (ELISA) by using DuoSet ELISA kits (R&D Systems), according to the manufacturer's instructions. ET-1 was assayed using RnD's QuantiGlo assay (R&D Systems).

Statistical analysis

Data are presented as mean values ± s.e.m. Data were analyzed with GraphPad-Prism 5.0 (Graphpad Software), and differences were considered statistically significant at $P < 0.05$.

Results

Exendin-4 reduces migration of ovarian cancer cells through the GLP-1R

The main effects of GLP-1 analogs are achieved through binding to the GLP-1R. In SKOV-3 and CAOV-3 cell lines, GLP-1R receptor presence was shown in Western blot analysis. GLP-1R was also observed in ovaries of patients with adenocarcinoma (Fig. 1A).

In transwell-based migration assay, Exendin-4 attenuated the MCP-1 induced migration in both SKOV-3 and CAOV-3 cell lines (Fig. 1B and C). Exendin-4 (50 nM) was added into the upper well and invasiveness was reduced by 13% in SKOV-3 cells and by 11% in CAOV-3 cells. Pre-treatment of Exendin-4 for 24 h further attenuated the migration potential by 22% in SKOV-3 cells and by 29% in CAOV-3 cells. In addition, GLP-1R antagonist (50 nM) reversed the inhibitory effects induced by Exendin-4 in both cell lines.

Incretin effects on apoptotic and viability

To assess the influence of incretin drugs on apoptosis and cell viability, SKOV-3 and CAOV-3 cells were stimulated with 50 nM of Exendin-4 for 24 h with or without the pre-treatment with Exendin-4 for 24 h. In both analyzed cancer cell lines, Exendin-4 induced the activation of caspase 3/7 by 31% in SKOV-3 cells and by 38% in CAOV-3 cells. Pre-treatment with Exendin-4 further increased activation of caspases by 56% in
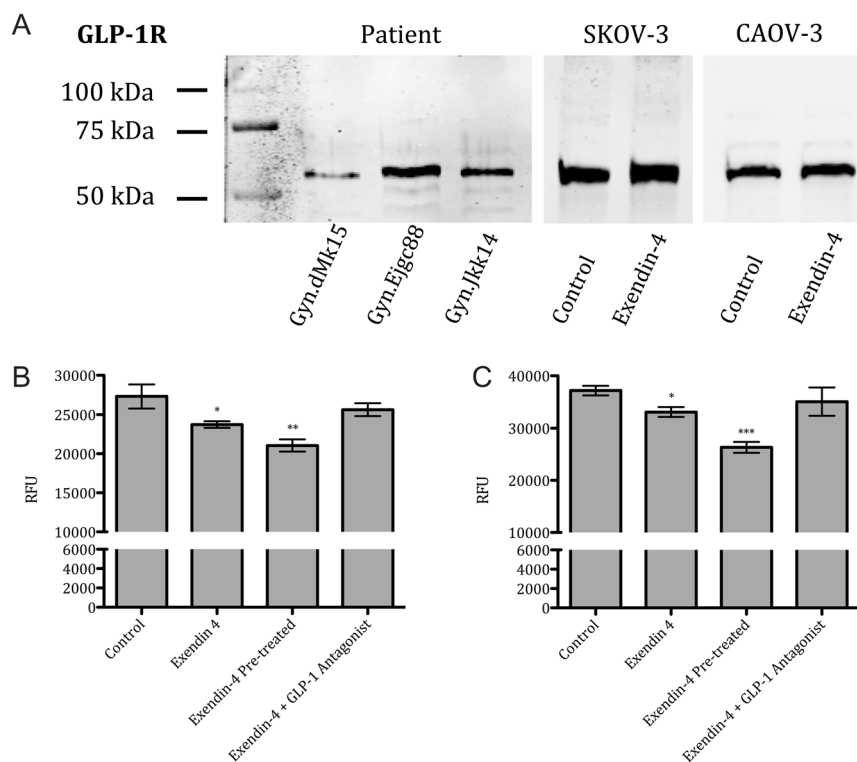
Figure 1

GLP-1R Western blot and transwell-based migration assay. Representative Western blot image of protein extracts from SKOV-3, CAOV-3 and patient biopsies (A). Ovarian cancer cell lines were stimulated for 24 h with Exendin-4 (50 nM), Exendin-4 (50 nM) pre-treatment for 24 h and GLP-1 Antagonist 9–36 (50 nM) in both SKOV-3 (B) and CAOV-3 (C). Relative fluorescence units (RFU). Mean values ± s.e.m. are shown. $n = 3$ per group. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ Control vs different conditions. One-way ANOVA followed with Dunnett's *post hoc*.

SKOV-3 cells and by 50% in CAOV-3 cells (Fig. 2A and D). Both cell lines displayed a similar pattern of viability.

In both cancer cell lines, stimulation with Exendin-4 with or without 24 h pre-treatment caused no significant changes in viability. Camptothecin (CPT) treatment caused a reduction in viability in SKOV-3 cells by 27% and by 28% in CAOV-3. Interestingly, Exendin-4 and CPT together further reduced viability of SKOV-3 cells by 44% and by 52% in CAOV-3. Additionally, cells pre-treated with Exendin-4 and incubated with Exendin-4 and CPT together caused a reduction in viability of SKOV-3 cells by 27% and by 38% in CAOV-3 (Fig. 2B and E).

In parallel, activation of GLP-1R signaling pathway was determined by multiplex analysis. Exendin-4 treatment decreased the phospho-IκB protein levels by 43% in SKOV-3 and by 36% in CAOV-3. Similarly, GLP-1 (100 nM) decreased the phospho-IκB protein levels by 40% in SKOV-3 and by 46% in CAOV-3 (Fig. 2C and F). The group treated with Camptothecin, a potent chemotherapeutic drug, also displayed a reduced pIκB/IκB ratio leading to an increased inhibition of NF-κB (24).

Distinct metalloproteinase production in Exendin-4-stimulated ovarian cancer cells

Ovarian cancer cells, SKOV-3 and CAOV-3, were incubated with TNF-α and incretin mimetic drugs and MMP levels were evaluated. MMP-1, MMP-2, MMP-7, MMP-9, MMP-10 and MMP-13 protein concentrations of cell culture mediums were assessed in both cancer cell lines upon stimulation with TNF-α, Exendin-4 and GLP-1 separately or in combination. Only detectable levels of MMPs were observed in the SKOV-3 cell line, whereas MMP levels were under detection limits for CAOV-3. After TNF-α incubation, treatment with either Exendin-4 or GLP-1 moderately decreased the protein level of all MMPs when compared with the TNF-α (Fig. 3). Upon TNF-α incubation, either Exendin-4 or GLP-1 caused an MMP-1 reduction by 14% and 19%, respectively (Fig. 3A). MMP-2 reduction was 23% and 47%, respectively (Fig. 3B). MMP-7 reduction was 22% and 32%, respectively (Fig. 3C). MMP-9 reduction was 15% and 21%, respectively (Fig. 3D). MMP-10 reduction was 16% and 37%, respectively (Fig. 3E). MMP-13 reduction by 27% was observed only when Exendin-4 was given (Fig. 3F). Interestingly, both
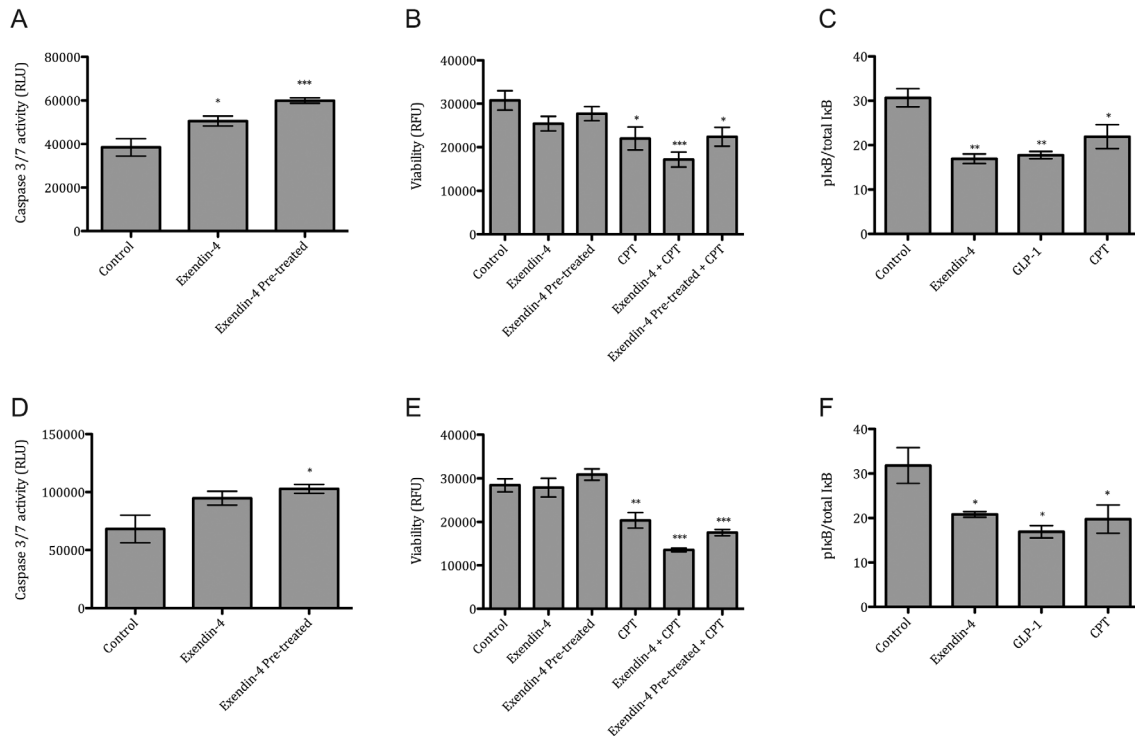
Endocrine Connections

Figure 2 Exendin-4 effects on apoptosis, cell viability and signaling pathway. Ovarian cancer cell lines were stimulated for 24 h with Exendin-4 (50 nM), Exendin-4 (50 nM) pre-treatment for 24 h and Camptothecin (CPT, 100 nM) in both SKOV-3 (A, B, C) and CAOV-3 (D, E, F). Caspase 3/7 activation in SKOV-3 (A) and CAOV-3 (D), cell viability in SKOV-3 (B) and CAOV-3 (E) and phosphoIκB in SKOV-3 (C) and CAOV-3 (F) was analyzed. Relative fluorescence units (RFU). Mean values ± s.e.m. are shown. $n = 3$ per group. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ Control vs different conditions. One-way ANOVA followed with Dunnett's *post hoc*.

Exendin-4 and GLP-1 caused a reduction in MMP-2 and MMP-9 protein levels in non-TNF-α groups when compared with the unstimulated group (Fig. 4B and D); this reduction is not observed for the other MMPs.

Exendin-4 modulates angiogenic factors and inhibits TNF-α endothelial cell apoptosis

We assessed the protein levels of VEGF in both cancer cell lines after stimulation with CPT and incretin agonists. VEGF protein levels were reduced in both cell lines when compared to the control group (Fig. 4). Exendin-4 caused a reduction by 14% and GLP-1 by 25% in SKOV-3 cells (Fig. 4A) and a reduction by 22% and GLP-1 by 19% in CAOV-3 cells (Fig. 4D). We also assessed the TNF-α in both cancer cell media after stimulation with CPT and incretin drugs. TNF-α protein levels were reduced in both cell lines when compared to the control group. Exendin-4 caused a reduction by 43% and GLP-1 by 50% in SKOV-3 cells (Fig. 4B), whereas in CAOV-3 cells Exendin-4 caused a reduction by 22% and GLP-1 by 21% (Fig. 4E). No changes were observed in inflammatory cytokines such as IL-6 and

IL-8 (Supplementary data 1, see section on supplementary data given at the end of this article).

Interestingly, endothelial cells from aorta, coronary artery and iliac artery showed differences in the expression levels of the GLP-1R (Fig. 4C). GLP-1R agonist treatment reduced the TNF-α-mediated apoptosis in iliac artery endothelium. Exendin-4 treatment at 1 nM or 10 nM reduced caspase 3/7 activation by 27% and 31%, respectively. GLP-1 treatment at 10 nM or 100 nM reduced caspase 3/7 by 32% and 30%, respectively (Fig. 4F).

Modulation of adhesion molecules, metalloproteinases and inhibitors of metalloproteinase production in endothelial cells after GLP-1 agonist treatment

The protein levels of selected adhesion molecules were quantified in TNF-α-stimulated endothelial cells (Fig. 5). Upon treatment with GLP-1R agonists (10 nM and 100 nM), only the higher concentration of either analog significantly reduced the levels of intracellular adhesion molecule 1 (ICAM-1) by 22% and 20%, respectively
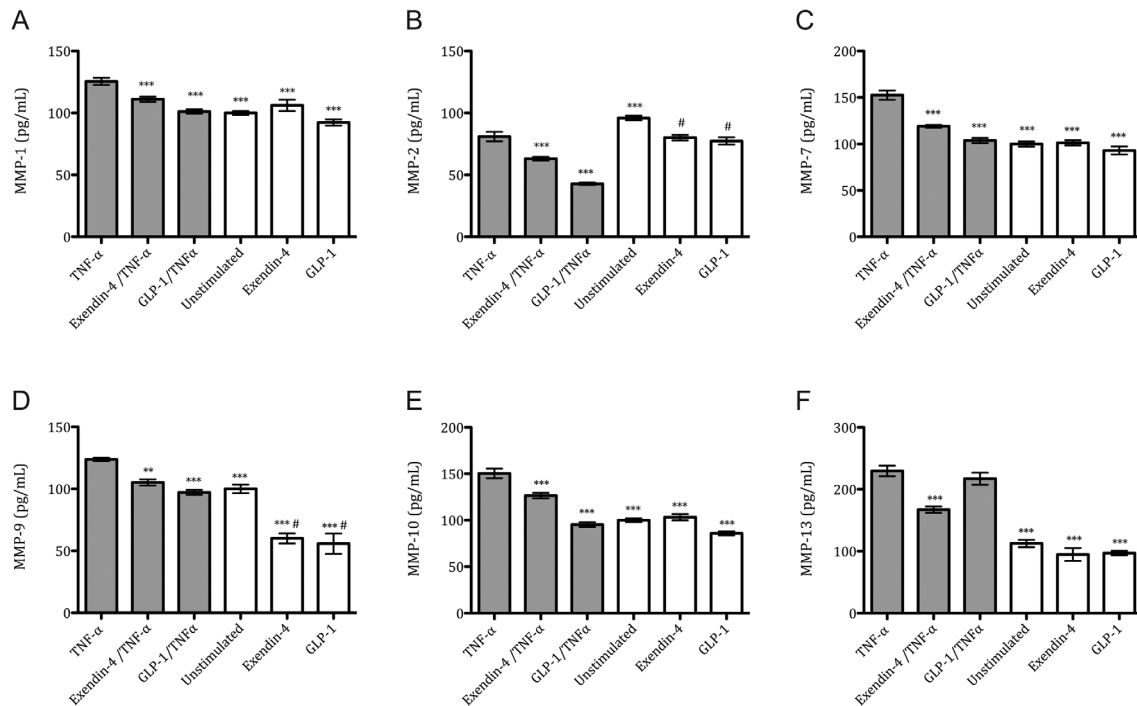
Figure 3 TNF-α-mediated metalloproteinase profile after Exendin-4 treatment in SKOV-3 cancer cells. Protein levels of MMP-1 (A), MMP-2 (B), MMP-7 (C), MMP-9 (D), MMP-10 (E) and MMP-13 (F) were analyzed by multiplex analysis after 24 h incubation with TNF-α (10 ng/mL), Exendin-4 (50 nM) and GLP-1 agonist (100 nM). Mean values ± s.e.m. are shown. $n = 3$–6 per group. Gray bars, TNF-α-stimulated. White bars, non-TNF-α-stimulated. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ TNF-α vs different conditions. #$P < 0.05$ Unstimulated vs Exendin-4 or GLP-1 group. One-way ANOVA followed with Dunnett's *post hoc*.

(Fig. 2A). Vascular adhesion molecule was also reduced by 50% and 43%, respectively (Fig. 2B). The levels of other adhesion molecules, P-selectin and Endothelin-1 (ET-1), remained unchanged (Supplementary data 2).

Endothelial cells in the presence of TNF-α treated with Exendin-4 at 10 nM only significantly reduced the levels of MMP-1 by 43% (Fig. 5C) and MMP-9 by 89% (Fig. 5D). Conversely, the levels of TIMP-1 and TIMP-2 (Fig. 5E and F), but not TIMP-3 (Supplementary data 2), were markedly increased in the higher concentration of both incretins. In the presence of TNF-α, Exendin-4 and GLP-1 caused an increase in TIMP-1 by 51% and 50%, respectively (Fig. 5E) and an increase in TIMP-2 by 65% and 52%, respectively (Fig. 5F).

Discussion

Evidence and epidemiological observational studies show an increase in the prevalence of both diabetes and cancer; thus, clinicians will have to face treatment of both conditions in the patient at the same time. In clinical environment, GLP-1 analogs and DDP-4 inhibitors are currently being used to treat T2D. Early data obtained from health register analysis and observational studies

have suggested that incretin drugs increase incidence of cancer, especially pancreatic cancer, in patients on incretin therapies (25). However, randomized clinical trials showed that incretin-based drugs were not associated with an increased risk of pancreatic cancer (26). Overall, the scarce *in vitro* data limit our understanding of the role of incretins and cancer. Although the mechanisms of action of GLP-1 analogs are yet to be elucidated, GLP-1 analogs have been shown to influence cancer cells by altering proliferation, apoptosis, ECM remodeling and the response to chemotherapy (17, 18, 19).

We studied several aspects of ovarian cancer progression such as apoptosis and migration, as well as metalloproteinase production associated during these processes. Consistent with the work of He and collaborators (19), we show that Exendin-4 suppresses ovarian cancer migration and induces apoptosis by the activation of GLP-1R in human ovarian cancer cells. Ovarian cancer patients are currently treated with platinum-based drugs and taxol (50); however, the results of this study indicate potential additive or synergic effect of incretin-chemotherapy treatment. Besides its anti-cancer effects, we observed anti-inflammatory effects, which are partially mediated by modulation of the NF-κB signaling pathway.
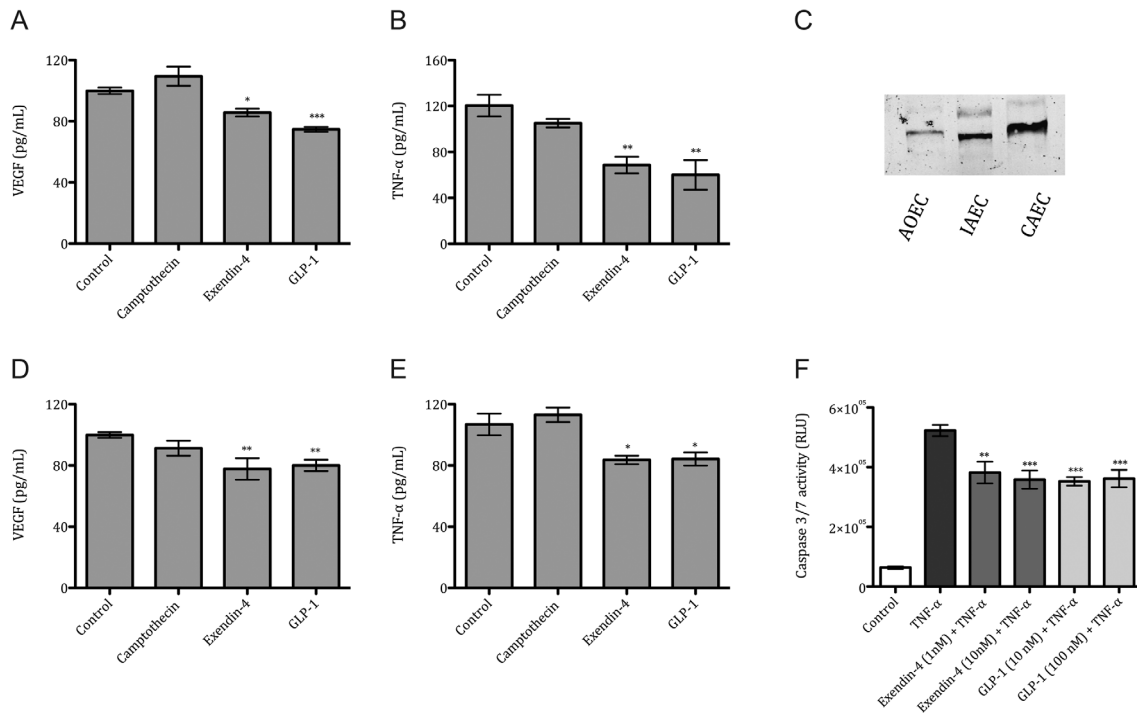
Figure 4 VEGF, TNF-α concentration in ovarian cancer cells and Caspase 3/7 activation in endothelium. VEGF protein levels in SKOV-3 (A) and CAOV-3 (D) and TNF-α protein levels in SKOV-3 (B) and CAOV-3 (E) after 24 h incubation with Camptothecin (100 nM), Exendin-4 (50 nM) and GLP-1 (100 nM). Representative Western blot image of GLP-1R expression in human Aortic Endothelial Cells (AoEC), Iliac Artery Endothelial Cells (IAEC) and Coronary Artery Endothelial Cells (CAEC) (C). Caspase 3/7 activation in TNF-α (10 ng/mL) stimulated IAEC after incubation with Exendin-4 (1 nM and 10 nM), and GLP-1 (10 nM and 100 nM) (F). Mean values ± s.e.m. are shown. $n = 3$–6 per group. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ TNF-α vs different conditions. One-way ANOVA followed with Dunnett's *post hoc*.

During early ovarian carcinogenesis, inflammatory mediators such TNF-α and TLR4 (27, 28) activate NF-κB signaling pathway by activation of the IKK complex, which then phosphorylates IκB. Phosphorylated IκB is then ubiquitinated and degraded by the proteasome system, leading to the release of NF-κB. The NF-κB molecule then translocates to the nucleus and initiates the transcription genes associated with metastasis, proliferation angiogenesis and suppression of apoptosis (29). We observed that treatment with Exendin-4 inhibits phosphorylation of IκB, hence the activation of the NF-κB signaling pathway.

Reasons for the high mortality rate associated with ovarian cancer include a late diagnosis, at which time cancer has metastasized throughout the peritoneal cavity. Ovarian tumor cells and the surrounding stromal cells stimulate the synthesis or activation of a family of proteolytic enzymes known as the MMPs to aid in tumor growth, invasion and eventual metastasis (12, 30). The MMP activity is controlled by their inhibitors, TIMPs. Thus, any disturbances in MMP/TIMP balance determine the type of tumor microenvironment which may facilitate metastasis (12). Studies have shown that MMP-9 overexpression is associated with an increased metastatic potential of ovarian tumors, which leads to poor prognosis and decreased survival (31). In another study, elevated levels of MMP-2 in serum were used as an indicator of the severity of invasion in breast cancer (32). Although the expression pattern of each individual MMP varies depending upon the type of tumor, tumor stage, patient diagnosis and even potentially the patient population (33), studies have shown that a reduction in MMP production reduces the metastatic potential of cancer cells (34). In this study, we have shown that incretin drugs can reduce the expression of several MMPs under TNF-α-mediated inflammatory environment in SKOV-3 cancer cell line. Furthermore, we were not able to assess detectable protein levels of MMPs in CAOV-3.

We studied the potential crosstalk between cancer cells and endothelial cells by analyzing the production of MMPs, cytokines such as VEGF, TNF-α, IL-6 by both cells types *in vitro*. These inflammatory mediators are recognized as an important component of the tumor–stroma interaction which may predispose to angiogenesis, invasiveness and increases in metastasis potential (35, 36, 37). We found that VEGF protein levels, a key molecule
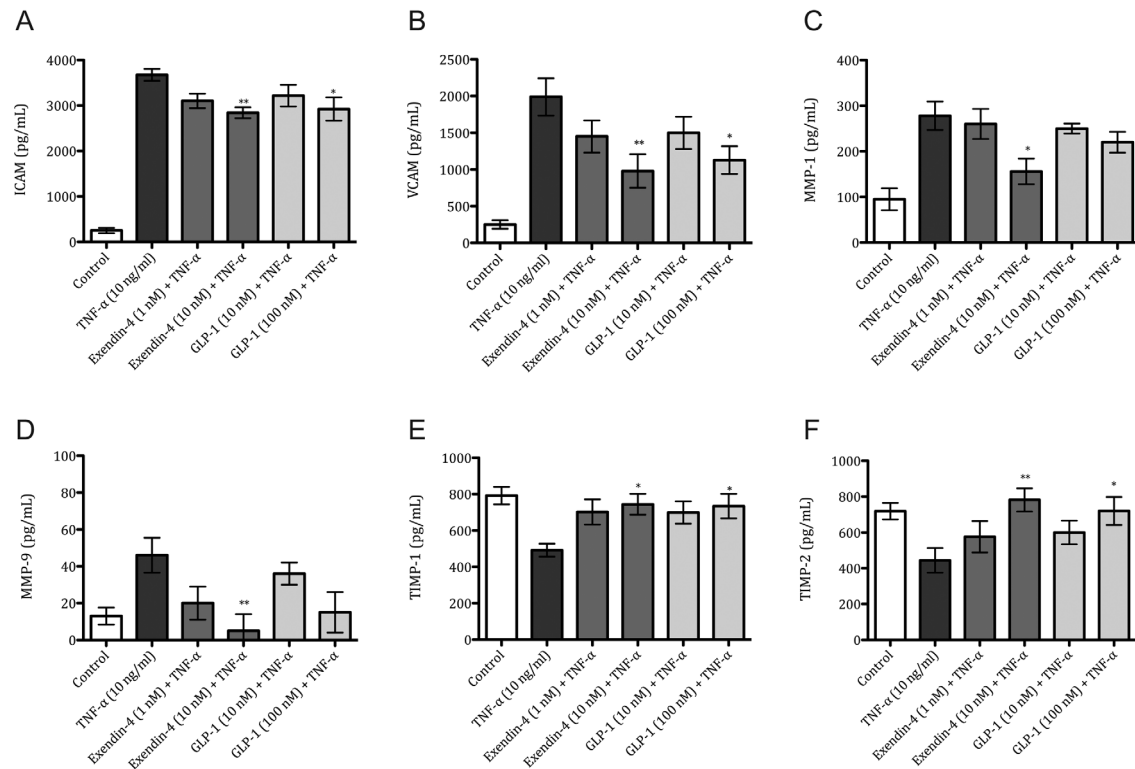
Endocrine Connections

Figure 5 Exendin-4 modulates endothelial-cell response after TNF-α stimulation. Iliac Artery Endothelial Cells (IAEC) were stimulated for 24 h with TNF-α (10 ng/mL), Exendin-4 (1 nM and 10 nM) and GLP-1 (10 nM and 100 nM). Protein concentration of VCAM-1 (A), ICAM-1 (B), MMP-1 (C), MMP-9 (D), TIMP1 (E) and TIMP-2 (F). Mean values ± S.E.M. are shown. *n* = 6 per group. *$P < 0.05$, **$P < 0.01$ TNF-α vs different conditions. One-way ANOVA followed with Dunnett's *post hoc*.

involved in neovascularization ([38], [39], [40]), were reduced in cancer cells after incretin drug treatment. Ovarian cancer inflammatory mediators such as TNF-α and IL-6, which are elevated in ovarian cancers ([41]), were also reduced upon incretin drug treatment.

Interestingly, recent investigations revealed a new mechanism by which cancer cells interact with endothelial cells during metastasis progression. In contrast to leukocyte transendothelial diapedesis, the research indicated that cancer cells can induce programmed cell death of the endothelial cells, hence promoting extravasation and metastasis ([42]). Through a series of *in vitro* experiments, the authors demonstrated that cancer cells can trigger the death of endothelial cells in a process mediated by TNF-α and MMPs ([43], [44]). In our study, we found that incretin drugs can reduce the TNF-α protein levels in both cancer cell lines, whereas the levels of other inflammatory cytokines were not affected. Furthermore, incretin drugs can influence vascular biology due to the presence of GLP-1R in endothelial cells. Consequently, in order to reproduce the effects that tumor cell may exert on endothelium at the moment of extravasation and metastasis, endothelial cells were stimulated with TNF-α

and treated with GLP-1R agonists. Contrarily to the effects in ovarian cancer cells, we have observed that incretin drugs prevent apoptosis of endothelial cells.

Ovarian cancer progression is characterized by the production of inflammatory mediators such as TNF-α and IL-1 ([45]). Studies have shown that these inflammatory molecules mediate the expression of endothelial adhesion molecules VCAM-1 and ICAM-1 ([46], [47]). Changes in the expression of cell adhesion molecules have been implicated in all steps of tumor progression, including migration of cells from primary tumor site to circulation, intravasation into the blood stream, extravasation to secondary metastatic places and formation of new tumors ([48]). Interestingly, we have shown that incretin agonists can reduce the expressions of both VCAM-1 and ICAM-1 adhesion molecules in endothelial cells suggesting a potential role of incretin drugs in reducing the metastatic potential of cancer cells. Additionally, production of metalloproteinases by endothelial cells is also a critical event during angiogenesis that occurs under normal and tumorigenic conditions ([49]). We observed a decrease in MMP-1 and MMP-9 production accompanied by an increase in the corresponding TIMPs.

Due to the hidden potential of incretin drugs as anti-cancer drugs, this study aimed to provide further understanding of the role of incretin drugs on tumor progression. Our work indicates that the pleiotropic effects of incretin drugs are not limited only to glucose-lowering actions, but incretins also modulate the actions of both ovarian cancer cells and endothelial cells involving several aspects of tumor progression. Although the synergic effects of anti-tumor and incretin therapy on tumor progression yet remain to be studied, incretin alone has shown great potential in the ovarian cancer field.

Supplementary data

This is linked to the online version of the paper at http://dx.doi.org/10.1530/EC-17-0294.

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.

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Endocrine Connections

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