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Endocrine glands-derived vascular endothelial growth factor protects pancreatic cancer cells from apoptosis via upregulation of the myeloid cell leukemia-1 protein

Li-Nan Ren [a,c], Qing-Fang Li [b], Feng-Jun Xiao [b], Jun Yan [b], Yue-Feng Yang [b], Li-Sheng Wang [b], Xiao-Zhong Guo [c,*], Hua Wang [b,*]

[a] State Key Laboratory of Cancer Biology and Institute of Digestive Diseases, Xijing Hospital of Digestive Disease, Fourth Military Medical University, Xi'an 710032, PR China
[b] Department of Experimental Hematology, Beijing Institute of Radiation Medicine, 27 Taiping Road, Beijing 100850, PR China
[c] Department of Gastroenterology, Shenyang General Hospital of PLA, 83 Wenhua Road, Shenyang 110016, PR China

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ABSTRACT

Endocrine glands-derived vascular endothelial growth factor (EG-VEGF, also termed as Prok1)—a novel cytokine that selectively acts on the endothelial cells of endocrine glands—was recently reported to be involved in the regulation of tumor cell growth and survival. However, its roles in the regulation of pancreatic cancer progression remain unclear. In this report, we investigated the suppressive effects of EG-VEGF on pancreatic cancer cell apoptosis and the relevant mechanisms. By using reverse-transcriptase polymerase chain reaction (RT-PCR) we found that the Mia PaCa II cells of the pancreatic cancer cell line express the mRNAs of both EG-VEGF (Prok1) and its receptors. EG-VEGF protects pancreatic cancer cells from apoptosis through upregulation of myeloid cell leukemia-1 (Mcl-1), an anti-apoptotic protein of the bcl-2 family. Treatment of pancreatic cancer cells with EG-VEGF results in the rapid phosphorylation of mitogen-activated protein kinase (MAPK), STAT3, and AKT, which are involved in the upregulation of Mcl-1 expression. EG-VEGF (Prok1) protects Mia PaCa II cells from apoptosis through G protein-coupled receptor (GPR)-induced activation of multiple signal pathways, and hence can be a novel target for pancreatic cancer therapy.

Introduction

Pancreatic cancer is the fourth leading cause of cancer-related mortality; approximately 200,000 people are diagnosed with pancreatic cancer each year throughout the world [1,2]. One of the well-known biochemical features of the pancreatic cancer cells is their resistance to apoptosis induced by various stimuli [3,4]. Thus, elucidation of the resistance mechanism of the pancreatic cancer cells to apoptosis can help in developing novel therapeutic strategies for the treatment of pancreatic cancer. Cytokine signals play important roles in the regulation of tumor cell apoptosis. LeCouter et al. [5] recently cloned an endocrine gland-derived vascular endothelial growth factor (EG-VEGF) encoding 305 amino acids. EG-VEGF exhibits high homology (80%) with a nontoxic protein purified from the venom of black mamba snakes, but low homology with the vascular endothelial growth factor (VEGF) [5]. EG-VEGF expression is high in steroidogenic tissues, including those of the pancreas, ovary, testis, adrenal gland, and placenta [5–9]. It is moderately expressed in nonsteroidogenic tissues such as the liver and colon, and in immune cells and inflamed human tissues [10–13]. EG-VEGF (Prok1) is a member of a novel family of secreted peptides, which includes Prok1 and Prok2, with multiple regulatory functions. EG-VEGF (Prok1) serves as a survival factor by modulating cell growth and survival, promoting angiogenesis [14], and regulating the contraction of gastrointestinal smooth muscle [15]. It promotes angiogenesis possibly through autocrine or paracrine mechanisms [12,16]. EG-VEGF expression has been identified in the human pancreatic gland and pancreatic cancer cells [9]; however, its effects on pancreatic cells are still unclear. The aim of this study is to investigate whether the aberrant EG-VEGF (Prok1) signaling pathways are involved in the resistance of pancreatic cancer cells to apoptosis and to elucidate the relevant mechanisms.

Materials and methods

Cells and culture conditions. The human pancreatic cancer cell line Mia PaCa II was purchased from the American Type Culture Collection. The cells were grown in Dulbecco's Modified Eagle

* Corresponding authors. Fax: +86 24 28856230 (X.-Z. Guo), +86 10 68158312 (H. Wang).
E-mail addresses: gxzsw@pub.sy.ln.cn (X.-Z. Guo), wanghua@nic.bmi.ac.cn, wanghualjh@gmail.com (H. Wang).

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Medium (DMEM; Cell Grow-Mediatech, Herndon, VA) containing 10% fetal bovine serum. The medium was supplemented with 2 mmol/L glutamine and 100 U/mL penicillin/streptomycin. Cell cultures were maintained under 5% CO_2 atmosphere at 37 °C.

Antibodies and reagents. EG-VEGF (Prok1) was purchased from Peprotech Inc. (Rocky Hill, New Jersey); anti-phospho-p44/42MAPK (where MAPK means mitogen-activated protein kinase), anti-p44/42MAPK antibody kit from Cell Signaling Technology (Beverly, MA); AKT (phospho-Ser473) antibody and STAT3 (phospho-Tyr705) antibody from Signalway Antibody (SAB); anti-human actin from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA); rabbit anti-human myeloid cell leukemia-1 (Mcl-1) antibody from R&D Systems, Inc. (Minneapolis, MN); PD98059, AG490, and Wortmannin from Calbiochem (San Diego, CA, USA); and pertussis toxin (PTX) from Biomol (Nottingham, UK). PD98059, AG490, Wortmannin, and PTX were completely dissolved in dimethylsulfoxide (DMSO) and used in experiments. Trizol Reagent was obtained from Invitrogen, Life Technologies (Grand Island, NY) and protein assay reagent was from Bio-Rad Laboratories (Hercules, CA).

Reverse transcription-polymerase chain reaction (RT-PCR). The total RNA was obtained using the Trizol method (Invitrogen Life Technologies) according to the manufacture's protocol. RNA was reverse transcribed using Reverse Transcriptase M-MLV(TaKaRa Biotechnology) and random primers were synthesized using the Takara Taq (Takara Bio) polymerase/buffer system. The sequences of PCR primers used for EG-VEGF were as follows: 5′-TGTGAGCGGG ATGTCCAGTGGGG-3′ (sense) and 5′-CTAAAAATTGATGTTCTTCAAG TCCATGG-3′ (antisense). β-Actin cDNA was used as the internal reference. After an initial denaturation step, 30 PCR cycles were performed (94 °C for 30 s, 54 °C for 1 min, and 72 °C for 1 min). PCR products were electrophoresed on 1% agarose gels and analyzed by ethidium bromide staining. The lengths of the EG-VEFG and β-actin bands were 215- and 307-bp, respectively.

Apoptosis assay. Apoptosis was measured using annexin-V/fluorescein isothiocyanate (FITC) apoptosis detection kit from BD PharMingen (San Jose, CA) and DAPI (4,6-diamidino-2-phenylindole) staining kit. About 0.3×10^6 cells were seeded in each well in the six-well plate. The cells were grown for 24 h in DMEM without fetal bovine serum, and without or with EG-VEGF (100 ng/mL). Both adherent and floating cells were collected, washed twice with cold phosphate-buffered saline (PBS), and resuspended in cold annexin-V binding buffer at a concentration of 1×10^7 cells/mL. Propidium iodide (PI) staining was performed to identify dead cells. Analysis was performed on a FACSCalibur flow cytometer (Becton Dickinson, San Jose, CA) using the Cell Quest software (Becton Dickinson).

The cells were seeded on the slides and treated as mentioned before. The slides were first washed with DAPI working buffer once, and subsequently, 0.5 mL of working buffer was added. The slides were then incubated at 37 °C for 30 min, rinsed once with methanol, and treated with buffer A. The morphology of the apoptotic cells stained with DAPI, annexin-V, and PI was analyzed by fluorescence microscopy.

Western blotting. Western blotting was preformed to analyze the levels of the phosphorylated and total forms of p44/42MAPK, AKT, STAT3, actin, and Mcl-1. The cells were washed with cold PBS and suspended in a lysis buffer (20 Mm Tris (pH 8.0), 150 mM NaCl, 1% NP-40, 1% deoxycholate, 1 mM EDTA containing 1 mM phenylmethylsulfonyl fluoride, 40 mM glycerophosphate, 125 μM Na_3VO_4, 50 mM NaF, 2 μg/mL eupeptin, 2 μg/mL aprotinin, 2 μg/mL pepstatin, and 1 mM dithiothreitol). Next, 30 μg of protein lysates were electrophoresed on a 10% sodium dodecyl sulfate (SDS)–polyacrylamide gel and then transferred to a Hybond-P polyvinylidene difluoride membrane. The blots were probed with specific antibodies for the abovementioned proteins and analyzed by enhanced chemiluminescence methods. It should be determined wherever the total cellular levels of phosphorylated or total p44/42MAPK, AKT, STAT3, actin, and Mcl-1 can be detected or not. The exposure time was minimized to avoid the saturation effects of these proteins due to their high abundance in the cells.

Results

EG-VEGF (Prok1) expression in Mia PaCa II cells

The expression levels of EG-VEGF mRNA in the Mia Paca II cells were determined using RT-PCR (Fig. 1A).

EG-VEGF (Prok1) protects Mia PaCa II cells from apoptosis

The Mia PaCa II cells were starved for 24 h, stained using annexin-V/PI (double-staining) and DAPI, and the cell morphology was analyzed by fluorescence microscopy. As shown in Fig. 1B and C, the apoptotic pancreatic cancer cells were identified by fluorescence microscopy; in the case of the cells that were serum-deprived, 27.74% were positive for annexin-V/FITC staining, whereas among the cells cultured in the presence of 100 ng/mL EG-VEGF, only 13.21% cells were positive for annexin-V/FITC staining (Fig. 1D).

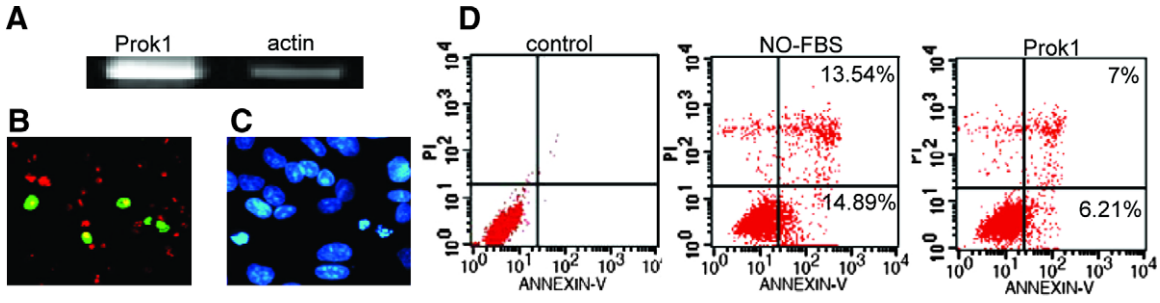

Fig. 1. The protective effects of EG-VEGF on Mia PaCa II cell apoptosis. (A) Expression of EG-VEGF in Mia PaCa II cells was analyzed by RT-PCR. β-Actin was used as the internal reference. The morphology of apoptotic cells was determined by fluorescence microscopy by using DAPI and annexin-V/PI staining. (B) Staining of apoptotic cells with annexin-V/PI. Unstained cells were classified as "live." Cells stained green with annexin-V were in the early apoptotic phase. Cells stained red with PI were considered to be dead. Cells stained with both annexin-V and PI were in the late apoptotic phase. (C) Staining of apoptotic cells with DAPI. The morphology of the cell nuclei was observed at an excitation wavelength of 350 nm by using a fluorescence microscope. If the nuclei are stained bright and homogenously, the cells were considered to possess a normal phenotype. If the chromatin at the periphery of the nuclear membrane appeared condensed or if the nuclear bodies exhibited a completely fragmented morphology, the cells were considered apoptotic. (D) Apoptosis detection by fluorescence microscopy. The apoptotic rate of cells cultured in the absence of fetal bovine serum is 27.74%, while that of the cells cultured in the presence of EG-VEGF is significantly low (13.21%, $p < 0.01$). These data represent the average values derived from three independent experiments. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

EG-VEGF triggers phosphorylation of extracellular kinase, AKT, and STAT3

We evaluated the phosphorylation of extracellular kinase (ERK), AKT, and STAT3 by EG-VEGF in Mia PaCa II cells. Treatment of Mia PaCa II cells with 100 ng/mL of EG-VEGF resulted in rapid phosphorylation of MAPK (indicated by labeled p42/44 MAPK in the figures), STAT3, and AKT. The phosphorylation signals were rapidly trigged within 20 min after treatment and were dose-dependent (Fig. 2A).

EG-VEGF upregulates Mcl-1 expression in Mia PaCa II cells

Mcl-1, which encodes an anti-apoptotic protein of the bcl-2 protein family, was originally identified as an early-induction gene during the differentiation of myeloid leukemia cells. Mcl-l plays a crucial role in cell division and malignant tumor development; this protein is over-expressed in a number of human malignancies, including human pancreatic carcinomas [17–19]. Since EG-VEGF demonstrated anti-apoptotic effects in Mia PaCa II cells, we subsequently investigated whether Mcl-1 is involved in EG-VEGF signaling. Mia PaCa II cells were starved overnight in DMEM without serum, and subsequently cultured in the absence or presence of 100 ng/mL of EG-VEGF for different periods (0, 0.5, 1, 2, and 6 h). The cultured cells were lysed and Mcl-1 expression was determined by Western blotting. The EG-VEGF-induced Mcl-1 upregulation in Mia PaCa II cells was observed to be time- and dose-dependent (Fig. 3A and B). Mcl-1 expression was transient, but peaked at 1 h post stimulation. Moreover, Mcl-1 expression was upregulated in cells treated with 25 ng/mL of EG-VEGF and was the highest in cells treated with 50 ng/mL of EG-VEGF. The EG-VEGF-induced upregulation of Mcl-1 is dependent on G protein-coupled receptor (GPR)-mediated MAPK, AKT, and STAT3 phosphorylation, which is inhibited by molecules that inhibit the GPR inhibitor, such as PTX (Fig. 3C).

EG-VEGF protects Mia PaCa II cells from apoptosis through by activation of multiple signals by GPR-induction

EG-VEGF protects the Mia PaCa II cells from apoptosis; however, the mechanism underlying this effect is still unclear. The roles of GPR in the activation of multiple signal pathways were investigated in this study. The cells were cultured overnight in DMEM without serum. Subsequently, the cells were pretreated with either the control media (0.01% DMSO) or with PTX (200 ng/mL), PD98059 (50 μM), AG490 (100 μM), or Wortmannin (10 μM) for 1 h, and then cultured with 100 ng/mL of EG-VEGF for 24 h. The cells were double stained with annexin-V/PI and the apoptotic cells were identified by flow cytometry. The apoptotic rate of Mia PaCa II cells cultured for 38 h under serum deprivation was 49.04%. In the presence of 100 ng/mL EG-VEGF, the apoptotic rate of Mia PaCa II cells was significantly less (35.21%). The apoptotic rate of the cells pretreated with PTX, PD98059, AG490, and Wortmannin—GPRs, MAPK, Stat3, and PI3K inhibitors, respectively—was noted to be higher than that of the control group ($p > 0.05$). These results indicated that protective effects of EG-VEGF on cells apoptosis are mediated through GPR-induced signals (Fig. 4).

Discussion

Programmed cell death, or apoptosis, plays a pivotal role in cellular homeostasis. Abnormal cells are eliminated by apoptosis; however, cancer cells overcome growth constraints and develop mechanisms to prevent apoptosis [4,20]. Autocrine growth factors play important roles in the prevention of tumor cells apoptosis. EG-VEGF is named so because of its unique and selective angiogenic effect in the endocrine glands. It has been reported that EG-VEGF promotes angiogenesis in the ovary and testis and induces the proliferation and migration of endothelial cells derived from the adrenal gland. However, unlike vascular endothelial growth factor (VEGF), EG-VEGF is a highly specific angiogenic mitogen that specifically regulates the vascular endothelium of endocrine gland, and has no effect on the endothelial cells derived from aorta, umbilical vein, or cornea [5].

In this report, we demonstrate that the EG-VEGF mRNA is expressed in the Mia PaCa II cells and that EG-VEGF protected the pancreatic cancer cells from apoptosis. Although the anti-apoptotic effect of EG-VEGF in other tissues has been reported [14,21], the mechanisms underlying this effect are poorly understood [22]. We also found that EG-VEGF can activate multiple signaling pathways by phosphorylating MAPK, phosphoinositide-3 kinase (PI3K), and JAK/STAT3 in a dose-dependent manner. These EG-VEGF-induced signal pathways also play important roles in the regulation of human neuroblastoma cells and capillary endothelial cells of the adrenal cortex [11,14,21]. Most importantly, EG-VEGF regulates the abovementioned three signal pathways that are involved in the apoptosis of pancreatic cancer cells and that are essential for the pancreatic cancer survival [23].

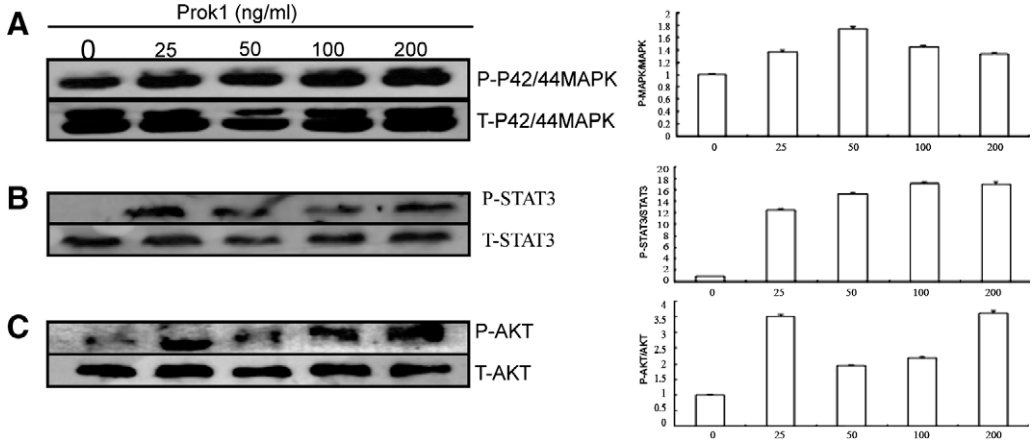

Fig. 2. Detection of phosphorylated MAPK, STAT3, and AKT by Western blotting. The cells were treated with different concentrations of EG-VEGF for 20 min and the cell lysates were assessed for MAPK, STAT3, and AKT activation. EG-VEGF modulated the activation of MAPK, STAT3, and AKT signaling pathways. The same blot was stripped and reprobed with antibody to MAPK, STAT3, and AKT, respectively, and used as a control of protein loading. The Western blot shown is a representative of at least three independent experiments.

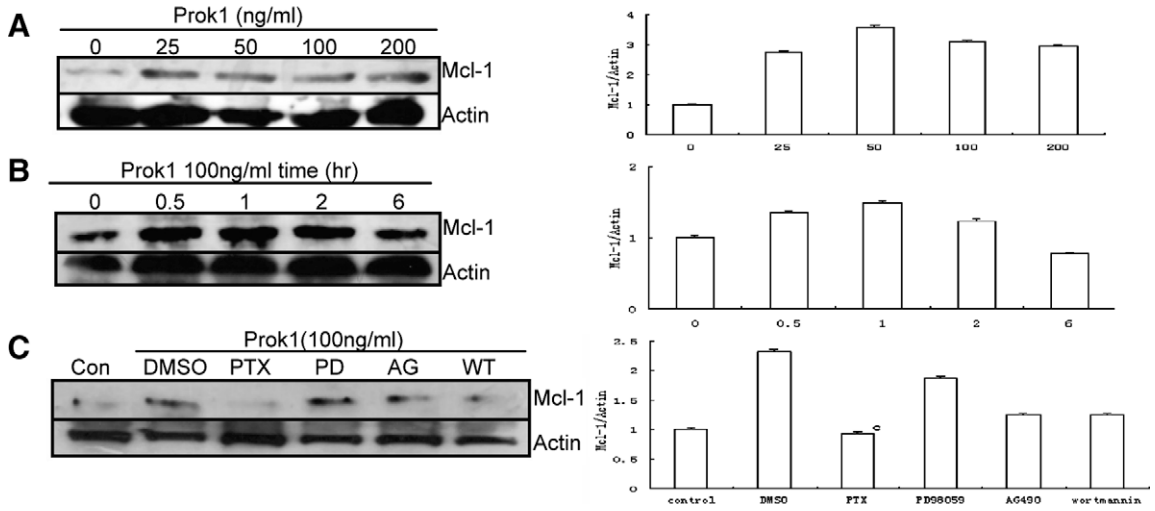

Fig. 3. EG-VEGF triggers dose- and time-dependent Mcl-1 expression in Mia PaCa II cells. (A) Concentration-dependent upregulation of Mcl-1 expression by EG-VEGF in Mia PaCa II cells. The cells were starved overnight in DMEM without serum, and subsequently cultured with various concentrations of EG-VEGF (0, 25, 50, 100, and 200 ng/mL); Mcl-1 expression in Mia PaCa II cells was upregulated in a dose-dependent manner after 1 h stimulation with EG-VEGF. Mcl-1 expression was upregulated at EG-VEGF concentration of 25 ng/mL and peaked at a concentration of 50 ng/mL. (B) EG-VEGF triggers time-dependent modulation of Mcl-1 expression in Mia PaCa II cells. Mia PaCa II cells were cultured overnight in DMEM without serum, and subsequently cultured with EG-VEGF (100 ng/mL) for different times (0, 0.5, 1, 2, and 6 h). Cells were lysed and Mcl-1 expression was determined by Western blotting. β-Actin served as the loading control. The time course of Mcl-1 expression shows that EG-VEGF-induced upregulation of Mcl-1 is transient and peaks at 1 h. β-Actin served as the loading control. (C) PD98059, AG490, Wortmannin, and PTX partly inhibited the EG-VEGF-induced upregulation of Mcl-1 expression. Regulation of Mcl-1 expression was analyzed using inhibitors of the Ras/ERK, Jak/STAT3, and PI3K/AKT pathways and the GPR inhibitor (PTX). Mia PaCa II cells were pretreated with either the control media (0.01% DMSO) or PTX (200 ng/mL), PD98059 (50 μM), AG490 (100 μM), or Wortmannin (10 μM) for 1 h before treatment with 100 ng/mL EG-VEGF. The cell lysates were obtained and Mcl-1 and actin expression was analyzed by Western blotting. The figure shows a representative of at least three independent experiments. The signal densities were determined by densitometry and were expressed as relative to that of the control, which was arbitrarily normalized to 1.

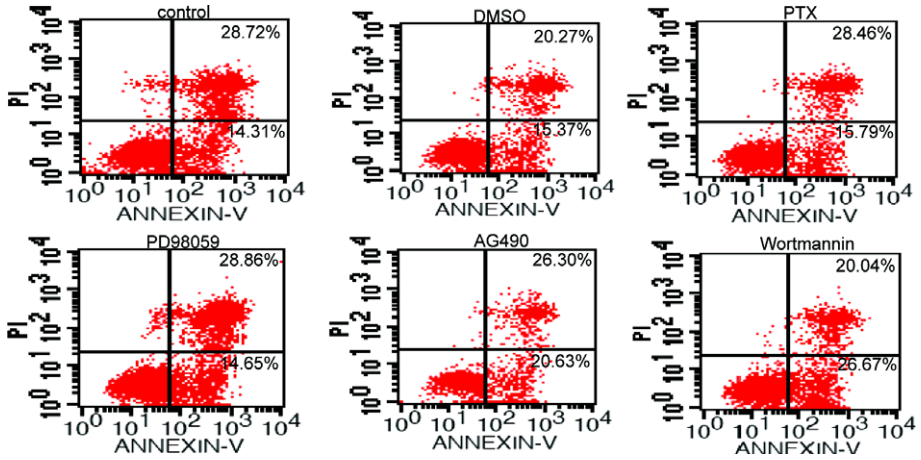

Fig. 4. The protective effect of EG-VEGF on apoptosis of pancreatic cancer cells can be inhibited by PTX and other inhibitors of the signal pathways. The cells were serum-starved overnight, and pretreated with PTX (200 ng/mL), PD98059 (PD, 50 μM), AG490 (AG, 100 μM), and Wortmannin (WT, 10 μM) or control (0.01% DMSO) for 1 h. The cells were washed with PBS and then cultured with or without 100 ng/mL EG-VEGF for 24 h. Apoptotic cells were identified by fluorescence microscopy. The data shown is from a single experiment and is representative of three independent experiments. The apoptotic rate of control cells cultured in the absence of serum for 38 h is 43.03%, while that of the cells cultured with EG-VEGF is significantly low (35.64%, $p < 0.01$); the apoptosis rate of cells pretreated with PTX, PD98059, PD98059, and Wortmannin is 44.25%, 43.51%, 46.93%, and 46.71%, respectively. The data represents the average values derived from three independent experiments.

Mcl-1 is an anti-apoptotic protein of the Bcl-2 protein family—a well established family of proteins—which significantly affects mitochondrial integrity, in that Mcl-1 influences the permeability of the mitochondrial membrane. Thus, Mcl-1 is an attractive target for novel gene therapy strategies in different carcinomas, including pancreatic cancer [24]. The anti-apoptosis mechanism of the pancreatic cancer cells is very elusive. In this study, we found that EG-VEGF functions as a survival cytokine in Mia PaCa II cells. EG-VEGF upregulates Mcl-1 expression in a dose- and time-dependent manner. Several signal pathways such as Ras/MAPK, PI3K/AKT, and Jak/STAT3 were involved in the upregulation of Mcl-1 expression [25]. We also investigated the roles of these signals in the EG-VEGF-induced upregulation of Mcl-1 expression. The results suggest that EG-VEGF activates the multiple signaling pathways, including MAPK, PI3K, and JAK/STAT3-induced pathways, in a dose-dependent manner. These three signaling pathways are involved in the apoptosis of pancreatic cancer cells [26]. EG-VEGF-induced Mcl-1 upregulation can partly be inhibited by specific inhibitors such as PD98059 (MER1/2 inhibitor), AG490 (a putative Jak2 inhibitor), and Wortmannin (PI3K inhibitor), indicating that these three signal pathways are involved in the apoptosis of pancreatic cancer cells.

As a member of the prokineticin family of proteins, EG-VEGF and its receptors (GPRs) influence many functions of different tissues [27,28]. PKR1 and PKR2 are the two GPRs belonging to the prokineticin family [8,29]. Although we have not identified which receptor plays a more important role, PTX was observed to attenuate the anti-apoptosis effect and upregulate the Mcl-1 protein

expression in Mia PaCa II cells. Thus, to enhance the anti-apoptotic effect, EG-VEGF binds to the GPRs; triggers the activation of MAPK, PI3K, and JAK/STAT3-PI3; and upregulates the expression of the anti-apoptotic protein Mcl-1. In conclusion, the findings of our study suggest that EG-VEGF protects the pancreatic cancer cells from apoptosis via upregulation of Mcl-1 and that multiple signal pathways are involved in this process. On the basis of these findings, novel targets for pancreatic cancer therapy can be explored.

Conflict-of-interest disclosure

The authors declare no competing financial interests.

Acknowledgments

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References

[1] S.L. Parker, T. Tong, S. Bolden, P.A. Wingo, Cancer statistics, CA Cancer J. Clin. 46 (1996) 5–27.
[2] G.J. Poston, J. Gillespie, P.J. Guillou, Biology of pancreatic cancer, Gut 32 (1991) 800–812.
[3] S. Mangray, T.C. King, Molecular pathobiology of pancreatic adenocarcinoma, Front. Biosci. 3 (1998) 1148–1160.
[4] H. Okada, T.W. Mak, Pathways of apoptotic and non-apoptotic death in tumor cells, Nat. Rev. Cancer 4 (2004) 592–603.
[5] J. LeCouter, J. Kowalski, J. Foster, P. Hass, Z. Zhang, L. Dillard-Telm, G. Frantz, L. Rangell, L. DeGuzman, G.A. Keller, F. Peale, A. Gurney, K.J. Hillan, N. Ferrara, Identification of an angiogenic mitogen selective for endocrine gland endothelium, Nature 412 (2001) 877–884.
[6] E.S.W. Ngan, K.Y. Lee, W.S.B. Yeung, H.Y.S. Ngan, Endocrine gland-derived vascular endothelial growth factor is expressed in human peri-implantation endometrium, but not in endometrial carcinoma, Endocrinology 147 (2007) 88–95.
[7] J. LeCouter, R. Lin, M. Tejada, G. Frantz, F. Peale, K. Hillan, N. Ferrara, The endocrine-gland-derived VEGF homologue Bv8 promotes angiogenesis in the testis: localization of Bv8 receptors to endothelial cells, Proc. Natl. Acad. Sci. USA 100 (2003) 2685–2690.
[8] T. Kisliouk, N. Levy, A. Hurwitz, R. Meidan, Presence and regulation of endocrine gland vascular endothelial growth factor/prokineticin-1 and its receptors in ovarian cells, J. Clin. Endocrinol. Metab. 88 (2003) 3700–3707.
[9] A. Morales, F. Vilchis, B. Chavez, C. Chan, G. Robles-Diaz, V. Diaz-Sanchez, Expression and localization of endocrine gland-derived vascular endothelial growth factor in human pancreas and pancreatic adenocarcinoma, J. Steroid. Biochem. Mol. Biol. 107 (2007) 37–41.
[10] T. Goi, M. Fujioka, Y. Satoh, Angiogenesis and tumor proliferation/metastasis of human colorectal cancer cell line SW620 transfected with endocrine glands-derived-vascular endothelial growth factor, as a new angiogenic factor, Cancer Res. 64 (2004) 1906–1910.
[11] Q. Li, B. Xu, L. Fu, X. Hao, Correlation of four vascular specific growth factors with carcinogenesis and portal vein tumor thrombus formation in human hepatocellular carcinoma, J. Exp. Clin. Cancer Res. 25 (2006) 403–409.
[12] M. Dorsch, Y. Qiu, D. Soler, N. Frank, T. Duonq, A. Goodearl, S. O'Neil, J. Lora, C.C. Fraser, PK1/EG-VEGF induces monocyte differentiation and activation, J. Leukoc. Biol. 78 (2005) 426–434.
[13] J. LeCouter, C. Zlot, M. Tejada, F. Peale, N. Ferrara, Bv8 and endocrine gland-derived vascular endothelial growth factor stimulate hematopoiesis and hematopoietic cell mobilization, Proc. Natl. Acad. Sci. USA 101 (2004) 16813–16818.
[14] R. Lin, J. LeCouter, J. Kowalski, N. Ferrara, Characterization of endocrine gland-derived vascular endothelial growth factor signaling in adrenal cortex capillary endothelial cells, J. Biol. Chem. 277 (2002) 8724–8729.
[15] M. Li, C.M. Bullock, D.J. Knauer, F.J. Ehlert, Q.Y. Zhou, Identification of two prokineticin cDNAs: recombinant proteins potently contract gastrointestinal smooth muscle, Mol. Pharmacol. 59 (2001) 692–698.
[16] A. Kaser, M. Winklmayr, G. Lepperdinger, G. Kreil, The AVIT protein family. Secreted cysteine-rich vertebrate proteins with diverse functions, EMBO Rep. 4 (2003) 469–473.
[17] Y. Miyamoto, R. Hosotani, M. Wada, J. Lee, T. Koshiba, K. Fujimoto, S. Tsuji, S. Nakajima, R. Doi, M. Kato, Y. Shimada, M. Imamura, Immunohistochemical analysis of Bcl-2, Bax, Bcl-X, and Mcl-1 expression in pancreatic cancers, Oncology 56 (1999) 73–82.
[18] C. Akgul, P. Turner, M. White, S. Edwards, Functional analysis of the human MCL-1 gene, Cell. Mol. Life Sci. 57 (2000) 684–691.
[19] K. Kozopas, T. Yang, H. Buchan, P. Zhou, R. Craig, MCL1, a gene expressed programmed myeloid cell differentiation, has sequence similarity to BCL2, Proc. Natl. Acad. Sci. USA 90 (1993) 3516–3520.
[20] I.M. Ghobrial, T.E. Witzig, A.A. Adjei, Targeting apoptosis pathways in cancer therapy, CA Cancer J. Clin. 55 (2005) 178–194.
[21] E.S. Ngan, F.Y. Sit, K. Lee, X. Miao, Z. Yuan, W. Wang, J.M. Nichools, K.K. Wong, M. Garcia-Barcelo, V.C. Lui, P.K. Tam, Implications of endocrine gland-derived vascular endothelial growth factor/prokineticin-1 signaling in human neuroblastoma progression, Clin. Cancer Res. 13 (2007) 868–875.
[22] E.S. Ngan, P.K. Tam, Prokineticin-signaling pathway, Int. J. Biochem. Cell Biol. 40 (2008) 1679–1684.
[23] X. Lin, S. Morgan-Lappe, X. Huang, L. Li, D.M. Zakula, L.A. Vernetti, S.W. Fesik, Y. Shen, 'Seed' analysis of off-target siRNAs reveals an essential role of Mcl-1 in resistance to the small-molecule Bcl-2/Bcl-XL inhibitor ABT-737, Oncogene 26 (2007) 3972–3979.
[24] I.T. Cavarretta, H. Neuwirt, G. Untergasser, P.L. Moser, M.H. Zaki, H. Steiner, H. Rumpold, D. Fuchs, A. Hobisch, J.A. Nemeth, Z. Nemeth, The antiapoptotic effect of IL-6 autocrine loop in a cellular model of advanced prostate cancer is mediated by Mcl-1, Oncogene 26 (2007) 2822–2832.
[25] Q.F. Li, C.T. Wu, Q. Guo, H. Wang, L.S. Wang, Sphingosine 1-phosphate induces Mcl-1 upregulation and protects multiple myeloma cells against apoptosis, Biochem. Biophys. Res. Commun. 371 (2008) 159–162.
[26] Y. Li, F.H. Sarkar, Inhibition of nuclear factor kappaB activation in PC3 cells by genistein is mediated via Akt signaling pathway, Clin. Cancer Res. 8 (2002) 2369–2377.
[27] P. Hoffmann, J.J. Feige, N. Alfaidy, Expression and oxygen regulation of endocrine gland-derived vascular endothelial growth factor/prokineticin-1 and its receptors in human placenta during early pregnancy, Endocrinology 147 (2006) 1675–1684.
[28] K. Urayama, C. Guilini, N. Messaddeq, K. Hu, M. Steenman, H. Kurose, G. Ert, C.G. Nebigil, The prokineticin receptor-1 (GPR73) promotes cardiomyocyte survival and angiogenesis, FASEB J. 21 (2007) 2980–2993.
[29] D.C. Lin, C.M. Bullock, F.J. Ehlert, J.L. Chen, H. Tian, Y.Q. Zhou, Identification and molecular characterization of two closely related G protein-coupled receptors activated by prokineticins/endocrine gland vascular endothelial growth factor, J. Biol. Chem. 277 (2002) 19276–19280.