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Exendin-4 inhibits growth and augments apoptosis of ovarian cancer cells☆

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ABSTRACT

Glucagon-like peptide (GLP)-1 promotes proliferation and survival in β-cell; however, whether GLP-1 receptor agonists promote growth of human ovarian cancer cells remain unknown. We aimed to explore the effects of GLP-1 agents on ovarian cancer cells. GLP-1 receptor expression in human ovarian cancer tissues was detected by immunohistochemical analysis. The effects of exendin-4, a GLP-1R agonist, were investigated on proliferation, migration and invasion, apoptosis in vitro and tumor formation in nude mice of ovarian cancer cells. Our study demonstrated that GLP-1R expressed in both human ovarian cancer tissues and cell lines. Exendin-4 inhibited growth, migration and invasion and enhanced apoptosis of ovarian cancer cells through inhibition of the PI3K/Akt pathway. And exendin-4 attenuated tumor formation by ovarian cancer cells in vivo. Our study suggests that GLP-1R agonists do not promote the growth of ovarian cancer and may even have anticancer effect on selected diabetic patients with ovarian cancer.

1. Introduction

Ovarian carcinoma is the leading cause of death from gynecological malignancy and epithelial ovarian cancer accounts for more than 90% of all malignant ovarian tumors (Siegel et al., 2013). Ovarian cancer accounts for about 4% of all female cancers (Anderson et al., 2004). The 5-year survival rates for patients remain poor (between 35 and 40%) (Beehler et al., 2006; Kikkawa et al., 2000). The main reason for the poor rate of survival is that most women are diagnosed at an advanced stage because of the absence of distinctive symptoms and specific tumor markers on early stages (Cannistra, 2004). Driven by obesity epidemic and the sedentary life style, there has been a continuous increase trend of incidence of type 2 diabetes throughout the world. Diabetes, accompanying or independent of obesity, is a well-known risk factor for both development and mortality from ovarian cancer (Coughlin et al., 2004; Diaz et al., 2013; Ma et al., 2013; Shah et al., 2014). The metabolic changes including insulin resistance, hyperinsulinemia, increased estrogen level and cytokine, and adipokine imbalances in diabetics likely contribute to an increase risk of malignancy as well as result in inferior cancer outcomes (Diaz et al., 2013; Howe et al., 2013). On the other hand, evidence has indicated that some antidiabetic agents may increase or decrease risk of cancer. Drugs that elevate insulin levels and induce weight gain may increase the risk of cancer; whereas drugs, such as metformin, that reduce insulin levels and lead to weight loss appear to decrease risk of cancer (Onitilo et al., 2012).

Recently, incretin therapy comprising GLP-1 receptor agonists are popular among clinicians and patients. It provides good glycemic control combined with low risk of hypoglycemia, improvement of lipid profile, weight loss, and insulin resistance (Forst et al., 2012). These benefits suggest GLP-1 may have a protective effect on ovarian cancer. As GLP-1 stimulates insulin secretion, sustained

Abbreviations: T2DM, type 2 diabetes mellitus; GLP-1, glucagon-like peptide -1; GLP-1 R, glucagon-like peptide -1 receptor; IHC, immunohistochemical; BSA, bovine serum albumin; c-AMP, cyclic adenosine monophosphate.

☆ The work was carried out in the First Affiliated Hospital of Sun Yat-sen University.

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Table 1
The association between GLP-1R expression and some clinical parameters.

Characteristics	Total	GLP-1R		P-value
		Positive	Negative	
Age				
≤45	48	7	41	0.42
>45	42	9	33	
Histological type				
Serous	48	8	40	0.69
Mucinous	21	3	18	
Others	21	5	16	
Tumor size				
≤4 cm	50	11	49	0.28
>4	40	5	35	
FIGO stage				
I+II	54	8	46	0.79
III+IV	36	8	28	
Grade				
1–2	51	7	44	0.41
3	39	9	30	
Lymph node metastasis				
Yes	32	4	28	0.56
No	58	12	46	

Others: clear cell, five cases; endometrioid, seven cases; transitional cell, six cases; mixed, three cases.

GLP-1R activation may, on the other hand, be associated with hyperinsulinemia. GLP-1R activation directly enhances cell proliferation and promotes cell survival in several tissues including β-cells, cardiomyocyte, fibroblasts, and neurons (Brubaker and Drucker, 2004). These data indicated GLP-1-based agents may increase risk of ovarian cancer. So clarifying the effect of GLP-1-based therapy on ovarian cancer has important clinical implications. In this study we aimed to explore the expression of GLP-1 receptor in ovarian cancer tissues and the effects of exendin-4 on growth, migration, invasion and apoptosis of ovarian cancer cells in vitro and in vivo.

2. Materials and methods

2.1. Patients and tumor specimens

The study was performed on material of 90 archival infiltrating ovarian cancers between January 2010 and January 2013 from Department of Pathology, the First Affiliated Hospital of Sun Yat-sen University. The median age was 48 years (range, 34–79 years). Histopathological diagnosis was based on WHO criteria, the samples were staged according to the International Federation of Gynecology and Obstetrics system and graded based on Gynecologic Oncology Group criteria. Clinical parameters were obtained by retrospective review of medical records. Review Board approval was obtained.

2.2. Immunohistochemical analysis

Immunohistochemical (IHC) reactions were performed on the total of 90 formalin fixed paraffin embedded ovarian cancer tissues for antibody GLP-1R (1:100,ab39072, Abcam, Cambridge, UK) as described (Jung and Kwon, 2014). To confirm specificity of anti-GLP-1R antibody, we also performed immunohistochemistry using other company's antibody (1; 50, NBP1–97308; Novus Biologicals, Littleton, CO). Twenty normal ovarian tissue samples from patients undergoing resection for non-oncological reasons were used as comparison. The intensity of the IHC reactions was independently evaluated by two pathologists. For the evaluation of GLP-1R expression, if reaction was observed in over 10% of cancer cells it was classified as positive.

2.3. Cell lines

Human ovarian cancer cell lines (SKOV3, OVCAR3, OVCAR4, A2780 and ES-2) were used, and cultured in DMEM or RPMI 1640

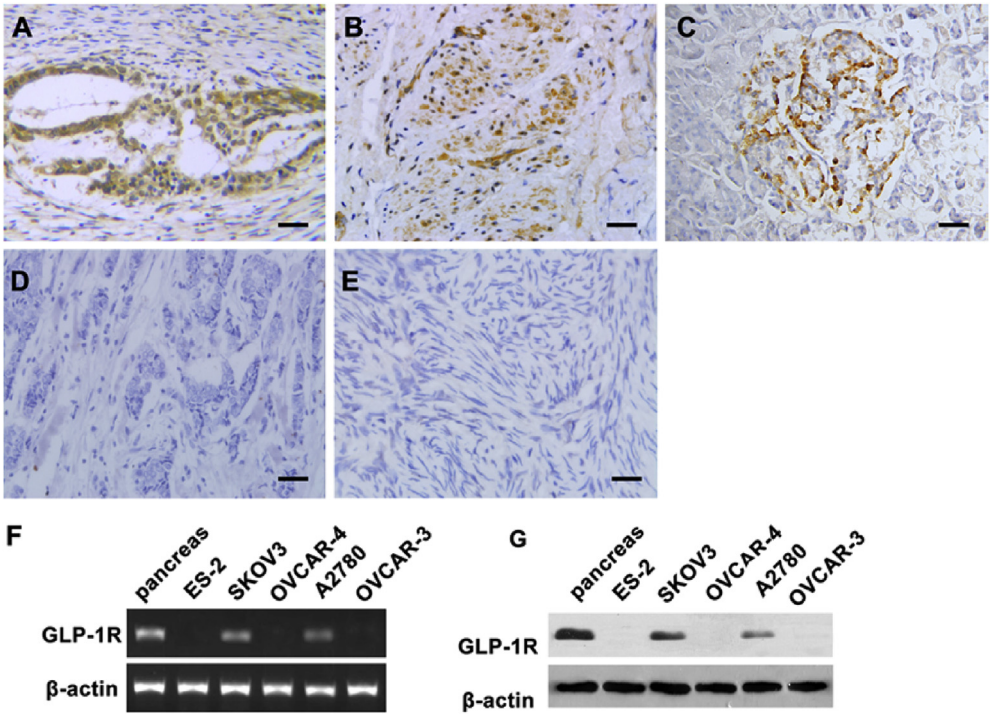

Fig. 1. GLP-1R expression in human ovarian cancer and normal tissue and in cell lines. GLP-1R expression in tissue sample was detected by immunohistochemical analysis. Positive GLP-1R staining in ovarian cancer tissue (serous ovarian adenocarcinoma, stage II) (A), normal ovarian tissue (B) and human pancreas tissue (C), negative GLP-1R staining in ovarian cancer tissue(D) and normal ovarian tissue (E),bars = 20 μm. RT-PCR and Western blot were performed to detect mRNA (F) and protein (G) levels of GLP-1R in ovarian cancer cells.