Int J Clin Exp Med 2016;9(2):1603-1611
www.ijcem.com /ISSN:1940-5901/IJCEM0016646

Original Article

Insulin analogs detemir, glargine and lispro enhance proliferation of human thyroid and gastric normal or cancer cell lines

Wei Cui[1], Mei-Ju Ji[2], Huan Chen[1], Ning Wang[1], Yu-Jing Zhao[1], Ying-Ying Liu[1], Bing-Yin Shi[1]

[1]*Department of Endocrinology, The First Affiliated Hospital of Xi'an Jiaotong University, Xi'an 710061, China;*
[2]*Center for Translational Medicine, The First Affiliated Hospital of Xi'an Jiaotong University, Xi'an 710061, China*

Received September 22, 2015; Accepted December 6, 2015; Epub February 15, 2016; Published February 29, 2016

Abstract: Previous studies indicate that insulin associated signals can activate the phosphatidylinositol 3-kinase (PI3K) and mitogen-activated protein kinase (MAPK) pathways, resulting in metabolic and mitogenic effects. The purpose of our study was to investigate the influence of the insulin analogs Detemir, Aspart, Glargine and Lispro on the human cancer cell cultures. We incubated thyroid cell line HTori3; thyroid cancer cell lines K1, 8305c, FTC133, normal gastric cell line GES-1, and gastric cancer cell lines MGC803 SGC7901 BGC823 with insulin, insulin-like growth factor-1 (IGF-1) or insulin analogs Detemir, Aspart, Glargine and Lispro, and assessed proliferation by MTT assay, apoptosis by flow cytometry and cell invasion with transwell cultures. In addition, phosphorylation of signaling molecules was detected by immunoblot. Detemir, Glargine and Lispro induced proliferation of K1 and HTori3, but had no effect on cell invasion of 8305c or SGC7901 cells. These insulin analogs varied in their capacity to induce proliferation in these cell lines, but all compounds increased the rate of apoptosis in all cell lines. ERK phosphorylation was not observed in response to insulin, IFG-1 or Glargine in GES-1, MGC803, BGC823 or HTori3 cells, however was observed in response to insulin, IFG-1 or Glargine in K1 and FTC133. Enhanced Akt phosphorylaition was not observed in any condition. Insulin analogs Detemir, Glargine and Lispro, but not Aspart, induce proliferation of normal and cancerous thyroid and gastric cell lines. The enhancement of proliferation mediated by insulin analogs in thyroid cancer cell lines may be mediated by ERK phosphorylation.

Keywords: Insulin, insulin-like growth factor-1, insulin analogs, PI3K-AKT pathway

Introduction

Patients with type 2 diabetes are known to have a modestly increased risk of developing cancer, and/or worse cancer prognosis than individuals without diabetes [1]. A recent survey conducted in Taiwan indicated that the risk of gastric cancer may be elevated by 14% in diabetic patients [2], and increased incidence of thyroid cancer has been reported in diabetic women [3]. Furthermore, recently the incidence of colorectal cancer in patients with type 2 diabetes was found to be elevated by hyperinsulinaemia and hyperglycemia.

Administration of exogenous insulin is the predominant treatment for type 1 and type 2 diabetes [4]. However, insulin-related signaling pathways have been implicated in tumor development [5], and the impact of long term insulin use on the incidence, development of metastasis of tumors are poorly defined.

Point mutations within the insulin gene have facilitated development of a range of short-acting and long-acting insulin analogs, that mimic physiological postprandial secretion and basal insulin secretion, respectively [6, 7]. Although the pharmacological profile of these analogs has been well characterized [8], the potential mitogenic effects of most of these analogs are yet to be systematically evaluated [9, 10].

Most insulin analogs were created by modifying the C-terminal of the native insulin β-chain, a region that does not participate in binding to the insulin receptor (IR) [7]. Short-acting insulin analogs Aspart and Lispro were generated

by exchanging B28 lysine with Aspartate, (Humalog, Eli Lilly, USA) and reversal of the penultimate lysine and proline residues, respectively. Long-acting insulin analog Detemir was generated by omitting a threonine residue at position B30, and acylation of a lysine residue at position B29 with myristic acid, a C14 fatty acid chain. Glargine (Lantus, Sanofi Aventis, Germany), also a long-acting insulin, was generated by substitution of asparagine with glycine at N21 and addition of two arginines to the carboxy terminal of the β-chain.

In addition to influencing absorption kinetics, some of these mutations have been found to enhance affinity for the insulin-like growth factor-1 (IGF-1) receptor (IGF-1R), which has been implicated in the putative carcinogenic activities of some of these compounds [11-13]. Although insulin is predominantly involved in the regulation of metabolism and IGF-1 is predominantly involved in growth processes, there is a certain degree of crosstalk between insulin, IGF-1 and their receptors [14, 15]. IGF-1R is a transmembrane tyrosine kinase receptor responsible for mediating most of the biological activities of IGF-1 and IGF-II. Insulin exhibits a number of IGF-1-like activities, such as growth stimulation, protection from apoptosis, and IGF-1 can exert certain metabolic effects [16]. In addition to mediating physiological growth and metabolic actions, stimulation of the IGF-1R has been implicated in tumor growth [14, 17], and IGF-1R expression has been found to be elevated in various neoplastic processes and many types of cancer [17]. IR and IGF-1R share 70% homology [18], and although IR is mainly involved in mediating metabolic activities, and IGF-1R primarily mediates cellular growth and differentiation [19], both receptors elicit putative carcinogenic activity in response to insulin binding [20].

Given the crosstalk between insulin and IGF-1 signaling pathways, and in view of the modifications introduced into the structure of insulin analogs, the widespread use of insulin and insulin analogs in recent years has raised a number of concerns among clinicians, patients and regulatory agencies, regarding the safety of the life-long use of these synthetic hormones.

Recent reports have highlighted the increased risk of malignant neoplasms and death in diabetic patients treated either with insulin or insulin analogs. The use of Glargine, in particular, has been associated with a possible increased risk of tumors in humans. However, in a Swedish study based on 114,841 diabetic patients and a Scottish study including 49,197 patients [21, 22], no relationship was found between administration of insulin analogs and cancer risk [23]. Hyperinsulinemia, in a non-obese mouse model of type 2 diabetes, has been reported to lead to increased mitogenic activity in follicular cells, incubation of follicular cells with TSH and insulin causes greater proliferation than incubation with TSH alone [24], suggesting that insulin may mimic the activity of IGF-1 in follicular cells. Follicular cells synthesize IGF-1 and express cell surface IGF-1 receptors, and this pathway as been implicated in the pathogenesis of thyroid nodules.

However, whether insulin is capable of inducing or promoting mitogenic effects through its cognate receptor or via the IGF-1R remains a controversial question [25-27]. Recently some insulin analogs have been reported to induce proliferation and inhibit apoptosis of human cell lines via activation of IGF-1-related signaling pathways [16, 28-30]. We sought to characterize and compare the capacity of insulin Detemir, Aspart, Glargine and Lispro to influence proliferation of cultured human gastric and thyroid cell lines, and to probe the mechanism by which these insulin analogs influence intracellular signaling. Our results indicate that in some cell lines, long-acting analog insulin Detemir induces proliferation via insulin-related signaling pathways in a similar fashion to IGF-1.

Materials and methods

Cell culture

The human thyroid cell line HTori3 and human gastric cancer cell lines MGC803, SGC7901, BGC823 were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS). The human thyroid cancer cell lines K1 and 8305c were cultured in Roswell Park Memorial Institute medium RPMI-1640 supplemented with 10% FBS, 1% glutamine, and 1% non-essential amino acid. The human follicular thyroid carcinoma cell line FTC133 was grown in Dulbecco's Modified Eagle Medium (DMEM)/F12 supplemented with 10% FBS. The human gastric cell line GES-1 was grown in DMEM with 10% FBS. Cells were incubated

Table 1. Screening of mammary epithelial cell lines for proliferative response to insulin

Cell lines	Proliferation (fold of control)
HToRi3	1.33±0.09
K1	1.20±0.05
8305c	1.02±0.07
FTC133	0.95±0.01
GES-1	0.89±0.04
MGC803	1.13±0.10
BGC823	0.95±0.02
SGC7901	1.12±0.04

Cells were starved for 24 h then incubated with 10 Mol insulin for 48 h. Proliferation was assessed using the MTT protocol. Proliferation was calculated by cell number in presence of insulin divided by cell number in the absence of insulin. Data presents mean ± SD.

with the following hormones and analogs: Insulin (Insulin R, Bayer); Detemir (Levemir, Novo Nordisk); Aspart (Novolog, Novo Nordisk); Glargine (Lantus, Sanofi Aventis); Lispro (Humalog, Eli Lilly) and IGF-1 (Peprotech American); Stock solutions of insulin and insulin analogs were made at 100 IU/mL and stock solutions of IGF-1 were made at 1 mg/mL.

Proliferation assay

Cells were plated in 96 well plates (1×10^4 cells/well) in complete medium. After 24 h the medium was of cancer cell lines was replaced with serum-free medium and the medium of normal cell lines was replaced with serum-reduced medium (containing 1% FBS). Hormones and analogs at the indicated concentrations were replenished every 24 h. After 48 h, proliferation was assessed by MTT assay. Briefly, 5 mg/ml MTT was added to plates, which were then incubated for 4 h. Supernatants were collected and absorbance was measured at 570 nm (Biotek, Synergy2, USA).

Apoptosis assay

Cells (2×10^5) were stained with PI and FITC-Annexin V (Sigma, USA) for 15 minutes and the percentages of apoptotic cells were analyzed by flow cytometry using BD FACSVerseTM (Becton & Dickinson Company, USA).

Cell invasion assay

Invasion of 8305C and SGC7901 cell lines was assessed using transwell chambers (Thermo, USA) with 8 mm pore polycarbonate filters coated with 50 mg/ml Matrigel™ (BD, Biosciences, Bedford, MA) diluted in serum-free medium. SGC7901 (10^5 cells/chamber) were seeded in the top of cylindrical cell culture inserts in serum-free RPMI-1640 media, while 8305c were cultures with 0.5% FBS. RPMI-1640 media with 10% FBS was placed in wells below and cells were incubated with 100 nM Detemir, IGF-1 or insulin for 24 h at 37°C in 5% CO_2. Non-migrating cells were removed from upper surfaces of chambers by scrubbing with a cotton swab. The cells on the lower membrane were fixed in 100% methanol for 20 min and stained with 0.1% Crystal Violet (Invitrogen) for 5 min at room temperature. Cells on the lower membrane were counted under a microscope.

Immunoblot

Cells were serum-starved overnight, then incubated with 10 nmol/L glargine, IGF-1 or insulin for 60 min. Cells were harvested with ice-cold PBS containing 5 mmol/L EDTA and lysed in a buffer composed of 150 mmol/L NaCl, 20 mmol/L Hepes, pH 7.5, 1% Triton X-100, 2 mmol/L EDTA, 2 mmol/L EGTA, 1 mmol/L PMSF, 2 μg/mL aprotinin, 1 mmol/L leupeptin, 1 mmol/L pyrophosphate, 1 mmol/L vanadate and 1 mmol/L DTT. Samples (150 mg) were subjected to 10% sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), followed by transfer of proteins to polyvinylidene fluoride membrane. Membranes were blocked with 5% bovine serum albumin (BSA), in T-TBS (20 mmol/L Tris-HCl, pH 7.5, 135 mmol/L NaCl and 0.1% Tween-20), then incubated with antibodies directed towards GAPDH (Abmart, China), phospho-ERK (Thr202/Tyr204, Bioworld, USA), phospho-AKT (Bioworld, USA). Membranes were then washed in TBST, incubated with horseradish peroxidase (HRP)-conjugated secondary antibody (ZSGB, China) for 1 h at room temperature and washed again in TBST. Protein signals were detected by enhanced chemiluminescence (ECL) and exposed to X-ray film. GAPDH was used as an internal control.

Statistical analysis

Data were expressed as mean ± SE. The Student's t-test (unpaired) was performed using InStat software version 3 (GraphPad Software, San Diego, CA). P values <0.05 were considered to be significant.

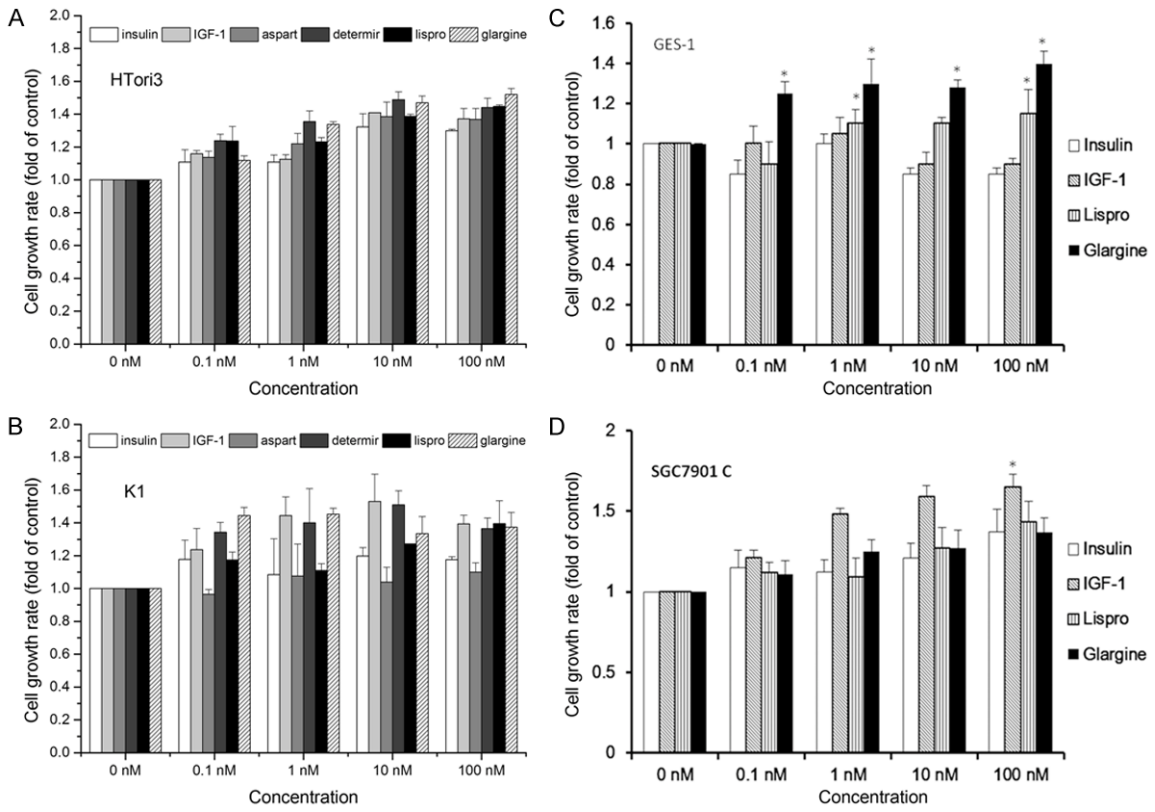

Figure 1. Dose-dependent impact of insulin, IGF-1 and insulin analogs on proliferation of (A) HTori3, (B) K1, (C) GES-1 and (D) SGC7901 cell lines. Cells were starved for 24 h, then treated with increasing concentrations of the indicated reagents for 48 h. Thereafter, proliferation was assessed using MTT assay. Proliferation is shown as fold of control (untreated cells). Values represent the mean ± SD. *P<0.05, **P<0.01.

Results

Insulin analogs induce proliferation of thyroid cell lines K1 and HTori3

We assessed the impact of insulin on proliferation of the normal thyroid cell line HTori3; gastric cancer cell lines MGC803, SGC7901, BGC823; the thyroid cancer cell lines K1 and 8305c; the human follicular thyroid carcinoma cell line FTC133 and the human normal gastric cell line GES-1. 10 nMol insulin induced mild proliferation in the normal thyroid cancer cell line, the two thyroid cancer cell lines tested and two of three gastric cancer cell lines tested, but neither the follicular thyroid carcinoma cell line nor human normal gastric cell line (**Table 1**). In the presence of insulin the thyroid cell line HTori3 grew 1.33±0.09 fold faster than the control, and the thyroid cancer cell line K1 grew 1.20±0.05 fold faster than the control. We further assessed the mitogenic capacity of insulin analogs in HTori3, K1, GES-1 and SGC7901 cells (**Figures 1** and **2**).

The enhancement of proliferation mediated by insulin and insulin analogs was more substantial in the thyroid cancer cell line, K1, than the normal thyroid cell line HTori3. Proliferation of HTori3 was enhanced more substantially by IFG-1 than insulin at concentrations above 10 nM (**Figure 1A**), and in the presence of 1 nM Aspart and Glargine and 10 nM Detemir and Lispro, increasing in a dose dependent manner. While the influence of insulin on K1 proliferation was only modest, after 48 hours, IGF-1 enhanced K1 proliferation significantly more substantially at concentrations above 0.1 nM (**Figure 1B**). K1 proliferation was also accelerated in the presence of 0.1 nM Detemir, Lispro and Glargine, and increased in a dose dependent manner, however, even at 100 nM Aspart mediated only a very limited enhancement of K1 proliferation (**Figure 1B**). Proliferation of GES-1 cells was enhanced in a dose dependent

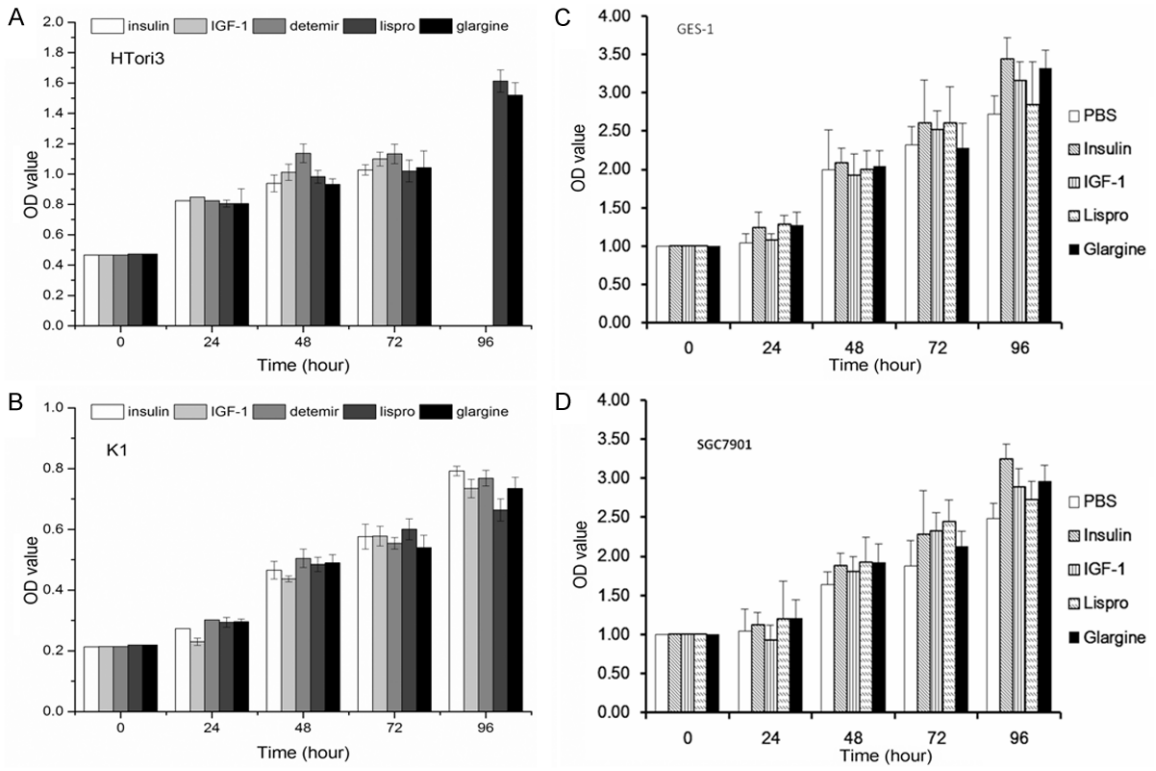

Figure 2. Time-dependent proliferative effects of insulin, IGF-1 and insulin analogs in (A) HTori3, (B) K1, (C) GES-1 and (D) SGC7901 cell lines. Cells were incubated with PBS or the indicated reagents at 10 nmol/L for indicated time. Thereafter, proliferation was assessed using MTT assay. Proliferation is shown as fold of control (untreated cells). Values represent the mean ± SD. *P<0.05.

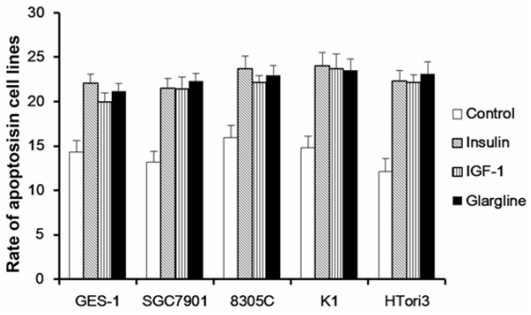

Figure 3. Rate of apoptosis in cell lines treated with insulin, IGF-1 or glargine. The level of apoptosis in GES-1, SGC7901, 8305C, Ki and HTori3 cells was assessed by flow cytometry in the presence of Insulin, IGF-1 and Glargine, but no significant differences in the level of apoptosis were detected between these three conditions.

manner with Glargine over 0.1 nM, and by Lispro over 1 nM. Proliferation of SGC7901C was enhanced only by Lispro over 100 nM.

In order to detect whether insulin analogs stimulate cellular proliferation in a time-dependent manner, serum-starved cell lines were incubated with insulin, IGF-I, Detemir, Lispro and Glargine (**Figure 2**). In all conditions HTori3, K1, GES-1 and SGC7901 cells proliferated within 72 h.

Insulin analogs induced apoptosis in cancer cell lines

The level of apoptosis in GES-1, SGC7901, 8305C, Ki and HTori3 cells was found to be elevated in the presence of Insulin, IGF-1 and Glargine, but no significant differences in the level of apoptosis were detected between these three conditions (**Figure 3**).

Insulin analogs did not alter invasive capacity of cancer cell lines

The migratory ability of human thyroid cancer cells has been reported to be rather low, thus invasion assays were performed in gastric cancer cells. The capacity of gastric cancer cell line SGC7901 and thyroid cancer cell line 8305C to

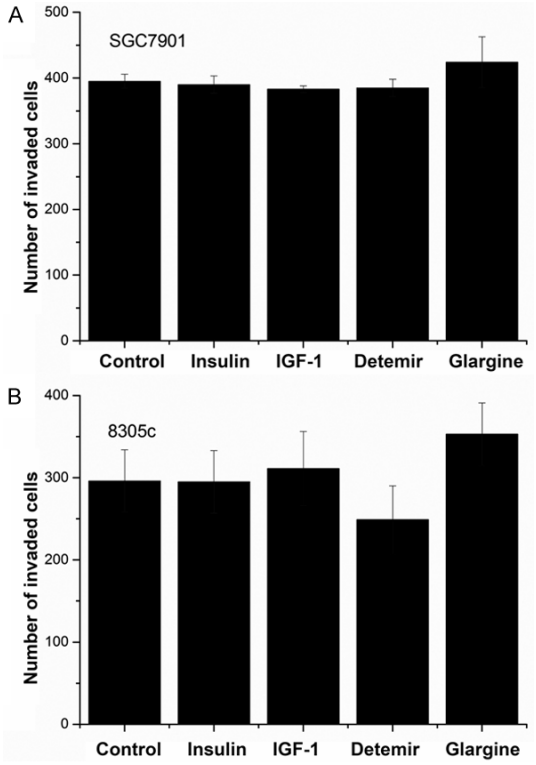

Figure 4. The influence of insulin analogs on cellular invasion. Invasive capacity of (A) SGC7901 and (B) 8305C incubated with PBS, insulin, IGF-1 or Glargine (100 nM). Five microscopic fields were arbitrarily chosen and invaded cells were counted. The results presented are average of three experiments carried out in triplicate and expressed as the mean ± SD.

cross a transwell insert was assessed following incubation with insulin, IGF-1 and insulin Detemir and Glargine, but no significant differences were observed in the number of cells crossing the filters (**Figure 4**).

ERK phosphorylation was involved in insulin Glargine- induced proliferation

The signaling pathways responsible for the cellular affects of insulin analogs remains to be determined. Both IR and IGF-1R are linked to two major signaling pathways: the ras-raf-MAPK and PI3K-PKB/AKT [11, 25, 26]. We assessed the activation of downstream signaling molecules by insulin, IFG-1 and insulin analog Glargine by immunoblot with anti-phospho-Akt and anti-phospho-ERK1/2 antibodies at 60 min. Increased ERK phosphorylation was not observed in response to any condition in GES-1, MGC803, BGC823 or HTori3 cells (**Figure 5A-F**), however was observed in response to insulin, IFG-1 and Glargine in K1 and FTC133 (**Figure 5E** and **5F**). Enhanced Akt phosphorylation was not observed in any condition.

Discussion

Insulin analogs facilitate less frequent dosing than insulin, however the modifications to these proteins may also influence their off-target biological activities, and safety concerns regarding the mitogenic potential of insulin analogs have been raised [31]. Follicular cells synthesize IGF-1 and express cell surface IGF-1 receptors, and this pathway as been implicated in the pathogenesis of thyroid nodules. We observed that both insulin and IGF-1 enhanced thyroid cancer and normal thyroid cell line proliferation. We found that long-acting insulin analogs Detemir and Glargine and short-acting insulin analog Lispro also enhanced proliferation of a thyroid cancer cell line, and that these insulin analogs, in addition to short-acting insulin analog Aspart, enhanced proliferation of a normal cancer cell line. However neither insulin nor IGF-1, nor the tested insulin analogs, impacted the invasion of the gastric cancer cell line SGC7901 and thyroid cancer cell line 8305C.

Previous studies have indicated that IGF-1R null cells cannot be transformed by several cellular or viral oncogenes, however they become susceptible to the oncogenic mediated transformation after the introduction of a functional IGF-1R [26, 32]. Insulin, unlike IGF-1, elicits mitogenic, rather than mutagenic effects, but can stimulate the growth of transformed cells, facilitating their escape from immune surveillance and thus increasing the incidence of clinically evident tumors [33, 34].

However, in vitro studies of the biological activities of insulin or insulin analogs have reported conflicting results, largely due to methodological differences between the study settings and designs [35]. We studied only cell lines with substantial expression of IR and IGF-1R. In thyroid cancers elevated expression of hybrid IR/IGF-IR receptors have been observed both in well-differentiated papillary carcinomas and in poorly differentiated/undifferentiated carcinomas, probably as a consequence of increased IR expression [36].

Long-acting insulin Detemir stimulated proliferation of thyroid cell line HTori3 and thyroid malignant cell line K1. Although the prolifera-

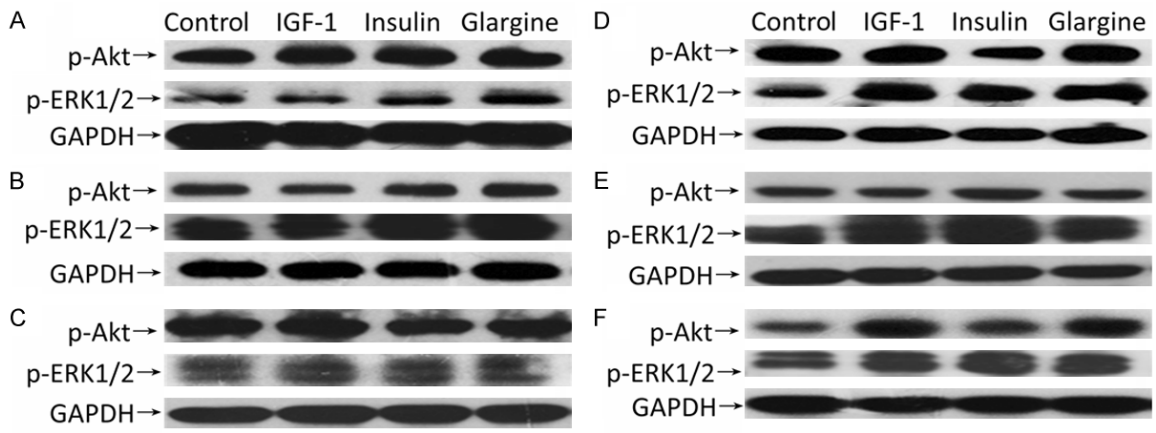

Figure 5. Activation of Akt and ERK by Glargine in GES-1 (A), MGC803 (B), BGC823 (C), HTori3 (D), K1 (E) and FTC133 (F). Cells were starved for 24 h and then incubated with PBS, IGF-1, insulin, or Glargine at 10 nmol/L for 60 min. Phospho-Akt and phospho-ERK1/2 were assessed using immunoblot.

tive potency of insulin was lower than Detemir and IGF-1, consistent with a recent study reporting that both insulin and Detemir significantly enhanced 3[H] thymidine uptake and MTT-assayed proliferation of normal human breast epithelial cells and breast cancer cells [29]. These ligands likely promote cell proliferation through multiple mechanisms, including prolonged stimulation of the insulin receptor, stimulation of the IGF-1R, and activation of ERK rather than PKB/AKT intracellular pathways. Indeed we observed no phosphorylation of AKT in response to insulin, IGF-1 or insulin analogs in any tested cell line.

We observed that long-acting insulin analogs Glargine and short-acting analog Lispro stimulated cell proliferation of thyroid cancer cells. Glargine has been previously reported to influence cell cycle progression and inhibit apoptosis [28, 29]. These observations indicate that the causal relationship between Glargine and gastric and thyroid cancers may be difficult to define, and ERK phosphorylation was not observed in response to insulin, IFG-1 or Glargine in GES-1, MGC803, BGC823 or HTori3 cells, but was observed in response to insulin, IFG-1 and Glargine in K1 and FTC133 cells.

In conclusion, the results presented here indicate that long-acting analogs Detemir and Glargine and short-acting analog Lispro can stimulate proliferation in human thyroid cancer cell lines, but cannot induce invasion of gastric cancer or thyroid cancer cell lines. However, our conclusions are limited by the number of cell lines we used, which cannot fully recapitulate the activity of these cell types in vivo. We were also unable to determine the mechanism of action responsible for this proliferative stimulation. Further studies are required to evaluate the signal transduction elicited by insulin analogs and the impact of these synthetic hormones in cellular physiology.

Acknowledgements

The authors wish to express their gratitude to Novo Nordisk, Bayer Company for giving free insulin and insulin analogs; we thank Department of Endocrinology 1st Affiliated Hospital of Xi'an Jiaotong University for applying cell lines, the experimental instructions and reagents.

Disclosure of conflict of interest

None.

Address correspondence to: Wei Cui and Bing-Yin Shi, Department of Endocrinology, The First Affiliated Hospital of Xi'an Jiaotong University, Xi'an 710061, China. Tel: +86-21-64879183; Fax: +86-21-64085875; E-mail: weicuiendor@126.com (WC); shibingy@126.com (BYS)

References

[1] Giovannucci E and Michaud D. The role of obesity and related metabolic disturbances in cancers of the colon, prostate, and pancreas. Gastroenterology 2007; 132: 2208-2225.

[2] Tseng CH. Diabetes, insulin use, and gastric cancer: a population-based analysis of the

Taiwanese. J Clin Gastroenterol 2013; 47: e60-64.
[3] Shih SR, Chiu WY, Chang TC and Tseng CH. Diabetes and thyroid cancer risk: literature review. Exp Diabetes Res 2012; 2012: 578285.
[4] Bethel MA and Feinglos MN. Basal insulin therapy in type 2 diabetes. J Am Board Fam Pract 2005; 18: 199-204.
[5] Giovannucci E, Harlan DM, Archer MC, Bergenstal RM, Gapstur SM, Habel LA, Pollak M, Regensteiner JG and Yee D. Diabetes and cancer: a consensus report. CA Cancer J Clin 2010; 60: 207-221.
[6] Vajo Z, Fawcett J and Duckworth WC. Recombinant DNA technology in the treatment of diabetes: insulin analogs. Endocr Rev 2001; 22: 706-717.
[7] Hirsch IB. Insulin analogues. N Engl J Med 2005; 352: 174-183.
[8] Owens DR, Zinman B and Bolli GB. Insulins today and beyond. Lancet 2001; 358: 739-746.
[9] Gupta K, Krishnaswamy G, Karnad A and Peiris AN. Insulin: a novel factor in carcinogenesis. Am J Med Sci 2002; 323: 140-145.
[10] Stammberger I, Bube A, Durchfeld-Meyer B, Donaubauer H and Troschau G. Evaluation of the carcinogenic potential of insulin glargine (LANTUS) in rats and mice. Int J Toxicol 2002; 21: 171-179.
[11] Bornfeldt KE, Gidlof RA, Wasteson A, Lake M, Skottner A and Arnqvist HJ. Binding and biological effects of insulin, insulin analogues and insulin-like growth factors in rat aortic smooth muscle cells. Comparison of maximal growth promoting activities. Diabetologia 1991; 34: 307-313.
[12] Slieker LJ, Brooke GS, DiMarchi RD, Flora DB, Green LK, Hoffmann JA, Long HB, Fan L, Shields JE, Sundell KL, Surface PL and Chance RE. Modifications in the B10 and B26-30 regions of the B chain of human insulin alter affinity for the human IGF-I receptor more than for the insulin receptor. Diabetologia 1997; 40 Suppl 2: S54-61.
[13] Werner H, Weinstein D and Bentov I. Similarities and differences between insulin and IGF-I: structures, receptors, and signalling pathways. Arch Physiol Biochem 2008; 114: 17-22.
[14] Baserga R. The IGF-I receptor in cancer research. Exp Cell Res 1999; 253: 1-6.
[15] Dupont J and LeRoith D. Insulin and insulin-like growth factor I receptors: similarities and differences in signal transduction. Horm Res 2001; 55 Suppl 2: 22-26.
[16] Eckardt K, May C, Koenen M and Eckel J. IGF-1 receptor signalling determines the mitogenic potency of insulin analogues in human smooth muscle cells and fibroblasts. Diabetologia 2007; 50: 2534-2543.
[17] Werner H and Maor S. The insulin-like growth factor-I receptor gene: a downstream target for oncogene and tumor suppressor action. Trends Endocrinol Metab 2006; 17: 236-242.
[18] Jansson M, Hallen D, Koho H, Andersson G, Berghard L, Heidrich J, Nyberg E, Uhlen M, Kordel J and Nilsson B. Characterization of ligand binding of a soluble human insulin-like growth factor I receptor variant suggests a ligand-induced conformational change. J Biol Chem 1997; 272: 8189-8197.
[19] Yang YX, Hennessy S and Lewis JD. Insulin therapy and colorectal cancer risk among type 2 diabetes mellitus patients. Gastroenterology 2004; 127: 1044-1050.
[20] Gauguin L, Klaproth B, Sajid W, Andersen AS, McNeil KA, Forbes BE and De Meyts P. Structural basis for the lower affinity of the insulin-like growth factors for the insulin receptor. J Biol Chem 2008; 283: 2604-2613.
[21] Jonasson JM, Ljung R, Talback M, Haglund B, Gudbjornsdottir S and Steineck G. Insulin glargine use and short-term incidence of malignancies-a population-based follow-up study in Sweden. Diabetologia 2009; 52: 1745-1754.
[22] Colhoun HM and Group SE. Use of insulin glargine and cancer incidence in Scotland: a study from the Scottish Diabetes Research Network Epidemiology Group. Diabetologia 2009; 52: 1755-1765.
[23] Currie CJ, Poole CD and Gale EA. The influence of glucose-lowering therapies on cancer risk in type 2 diabetes. Diabetologia 2009; 52: 1766-1777.
[24] Parameswaran R, Brooks S and Sadler GP. Molecular pathogenesis of follicular cell derived thyroid cancers. Int J Surg 2010; 8: 186-193.
[25] Kurtzhals P, Schaffer L, Sorensen A, Kristensen C, Jonassen I, Schmid C and Trub T. Correlations of receptor binding and metabolic and mitogenic potencies of insulin analogs designed for clinical use. Diabetes 2000; 49: 999-1005.
[26] Laron Z. Insulin-like growth factor 1 (IGF-1): a growth hormone. Mol Pathol 2001; 54: 311-316.
[27] LeRoith D, Goode J and Bock G. In Biology of IGF-1: It's Interaction with Insulin in Health and Malignant States. Chichester: John Wiley and Sons; 2004.
[28] Weinstein D, Simon M, Yehezkel E, Laron Z and Werner H. Insulin analogues display IGF-I-like mitogenic and anti-apoptotic activities in cultured cancer cells. Diabetes Metab Res Rev 2009; 25: 41-49.
[29] Shukla A, Grisouard J, Ehemann V, Hermani A, Enzmann H and Mayer D. Analysis of signaling pathways related to cell proliferation stimulat-

ed by insulin analogs in human mammary epithelial cell lines. Endocr Relat Cancer 2009; 16: 429-441.

[30] Yehezkel E, Weinstein D, Simon M, Sarfstein R, Laron Z and Werner H. Long-acting insulin analogues elicit atypical signalling events mediated by the insulin receptor and insulin-like growth factor-I receptor. Diabetologia 2010; 53: 2667-2675.

[31] Hemkens LG, Grouven U, Bender R, Gunster C, Gutschmidt S, Selke GW and Sawicki PT. Risk of malignancies in patients with diabetes treated with human insulin or insulin analogues: a cohort study. Diabetologia 2009; 52: 1732-1744.

[32] LeRoith D, Werner H, Beitner-Johnson D and Roberts CT Jr. Molecular and cellular aspects of the insulin-like growth factor I receptor. Endocr Rev 1995; 16: 143-163.

[33] Pollak M. The insulin and insulin-like growth factor receptor family in neoplasia: an update. Nat Rev Cancer 2012; 12: 159-169.

[34] Gallagher EJ and LeRoith D. Minireview: IGF, Insulin, and Cancer. Endocrinology 2011; 152: 2546-2551.

[35] Werner H and Chantelau EA. Differences in bioactivity between human insulin and insulin analogues approved for therapeutic use- compilation of reports from the past 20 years. Diabetol Metab Syndr 2011; 3: 13.

[36] Belfiore A, Pandini G, Vella V, Squatrito S and Vigneri R. Insulin/IGF-I hybrid receptors play a major role in IGF-I signaling in thyroid cancer. Biochimie 1999; 81: 403-407.