AMPK-dependent regulation of GLP1 expression in L-like cells

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Abstract

This study examined whether AMPK, an evolutionarily conserved sensor of cellular energy status, determines the production of glucagon-like peptide-1 (GLP1).

A negative relation existed between phosphorylation of AMPK α and the expression and secretion of GLP1 during changes in energy status in STC-1 cells, an L-like cell line. High concentration of glucose (25 mmol/L) decreased AMPK α phosphorylation, whereas it stimulated the expression and secretion of GLP1 relative to 5.6 mmol/L glucose. Serum starvation upregulated AMPK α phosphorylation, whereas it reduced GLP1 production significantly. Stimulation of AMPK phosphorylation by AICAR and overexpression of wild-type AMPK α 1, constitutively active AMPK α 1 plasmids, or AMPK α 1 lentivirus particles suppressed proglucagon mRNA and protein contents in STC-1 cells. Inactivation of AMPK by Compound C, AMPK α 1 siRNA or kinase-inactive AMPK α 1 mutant increased the expression and secretion of GLP1. Our results suggest that AMPK α 1 may link energy supply with the production of GLP1 in L-like cells.

Key Words

- ▶ enteroendocrine L cells
- ▶ GLP1
- ▶ energy sensing
- ▶ AMPK α 1

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Introduction

The proglucagon gene (*GCG*) encodes both glucagon and glucagon-like peptides (GLPs) in pancreatic α -cells and intestinal enteroendocrine L cells as well as neurons, respectively (Baggio & Drucker 2007). Glucagon is predominantly generated in pancreatic α -cells by prohormone convertase (PC)-2, whereas in the gut and brain, *GCG* expression leads to GLP1 synthesis after cleavage by PC-1/3 (Mojsov *et al.* 1986, Drucker & Asa 1988). The biologically active forms of GLP1 are GLP1(7–37) and GLP1(7–36)NH2 (Orskov *et al.* 1993). The half-life of bioactive GLP1 in the circulation is less than 2 min owing to rapid inactivation by the ubiquitous proteolytic enzyme dipeptidyl peptidase-4 (DPP4) (Deacon *et al.* 1995).

DPP4 rapidly converts GLP1(7–37) and GLP1(7–36)NH2 to their inactive metabolites GLP1(9–37) or GLP1(9–36)NH2 (Hansen *et al.* 1999). GLP1 secretion occurs primarily in response to nutrient ingestion, through a complex array of direct and indirect mechanisms (Baggio & Drucker 2007). Postprandial GLP1 levels peak within 15–30 min of nutrient consumption. GLP1, a potent antihyperglycemic hormone, induces glucose-dependent stimulation of insulin secretion, whereas suppresses glucagon secretion (Herrmann *et al.* 1995). Such glucose-dependent action is attractive particularly because when the plasma glucose concentration is in the normal fasting range, GLP1 no longer stimulates insulin release, thus avoiding

hypoglycemia (Elahi *et al.* 1994). Insulinotropic activity of GLP1 in type 2 diabetes mellitus, therefore, offers great potential for treatment of hyperglycemia without causing hypoglycemia (Elahi *et al.* 1994). Current GLP1-based therapies include long-acting GLP1 receptor (GLP1R) agonists (Buse *et al.* 2004) and inhibitors of GLP1 degradation (Aschner *et al.* 2006). With the introduction of GLP1-based treatments into the clinical practice, interest in factors that regulate the release of endogenous GLP1 has been heightened.

AMPK, which consists of a catalytic α -subunit and noncatalytic β - and γ -subunits, is a fuel sensor and regulator that plays a vital role in cellular energy homeostasis (Kim & Lee 2005). Enzyme activity is regulated both allosterically by AMP and by reversible phosphorylation at Thr-172 of the α -subunit by an upstream kinase (AMPK kinase or AMPKK) (Hardie *et al.* 1999). AMPK controls whole-body glucose homeostasis by regulating metabolism in multiple peripheral tissues such as skeletal muscle, liver, adipose tissues and pancreatic β -cells (De Morentin *et al.* 2011). By responding to diverse nutritional and hormonal signals, AMPK serves as an integrator of intertissue signals among peripheral tissues and the hypothalamus to control energy intake and whole-body energy balance (Minokoshi *et al.* 2004, De Morentin *et al.* 2011). The effects of AMPK on GLP1 synthesis in L cells remain largely uncharacterized.

Our previous studies demonstrate that mTORC1 enhances GLP1 synthesis (Xu *et al.* 2015a,b). As AMPK has been characterized to suppress mTOR signaling (Inoki *et al.* 2006), we hypothesize that AMPK contributes to the regulation of GLP1 production. Here, we present evidence that AMPK α 1 regulates the production of GLP1 in STC-1 cells. Our data identify AMPK as a critical mechanism regulating proglucagon gene expression and GLP1 secretion, thus expanding its interest as a potential target for the treatment of type 2 diabetes mellitus.

Materials and methods

Materials

Diprotin was from Sigma Chemical. Control lentiviral activation particles, AMPK α 1 lentiviral activation particles, Polybrene, control siRNA, AMPK α 1 siRNA and mouse anti-AMPK α 1 antibody were purchased from Santa Cruz Inc (Santa Cruz, USA). AICAR, mouse anti- β -actin, rabbit anti-AMPK α (Thr172) and rabbit anti-AMPK α antibodies were obtained from Cell Signaling Technology. Rabbit anti-AMPK α 2 antibody and mouse

anti-GLP1 were from Abcam. IRDye-conjugated affinity-purified anti-rabbit, anti-mouse IgGs were purchased from Rockland (Gilbertsville, PA, USA). TRIzol reagent and the reverse transcription (RT) system were from Promega. Lipofectamine was purchased from Invitrogen. Compound C and Glucagon-Like Peptide-1 Active enzyme immunoassay kit were purchased from Millipore.

Cell culture and transfection

The intestinal secretin tumor cell line (STC-1) was purchased from ATCC. All experiments were performed using passages 20–25. STC-1 cells were maintained in DMEM medium supplemented with 2.5% fetal bovine serum and 10% horse serum at 37°C in an atmosphere of 5% CO₂ air. For transient transfection, cells were plated at optimal densities and grown for 24 h. Cells were then transfected with the following constructs: GFP, wild-type AMPK α 1 (WT), constitutively active form of AMPK α 1 (AF), or the dominant negative form of AMPK α 1 (DN) plasmids (Corradetti *et al.* 2004, Inoki *et al.* 2006) using Lipofectamine reagent according to the manufacturer's instruction.

Infection of AMPK α 1 lentivirus

Lentiviral infection was performed according to the manufacturer's instruction. The infected STC-1 cells were seeded in a 6-well tissue culture plate and grown to about 80% confluency. A mixture of complete medium with Polybrene at a final concentration of 5 μ g/mL was prepared, and 3 mL of the mixture were added to each

Table 1 Anthropometric data (ESM).

Parameter	Lean	Obese	P value
Age (years)	52 $\pm$ 3.5	43 $\pm$ 12.7	0.545
Body weight (kg)	59 $\pm$ 2.2	99 $\pm$ 14.3*	0.04
BMI (kg/m ²)	23.3 $\pm$ 2.2	41.2 $\pm$ 7.8*	0.012
Fasting plasma glucose (mmol/L)	5 $\pm$ 0.2	8 $\pm$ 0.3*	0.023
HbA1c (%)	3.9 $\pm$ 0.3	7.6 $\pm$ 0.5*	0.013
Total cholesterol (mmol/L)	5.9 $\pm$ 1.3	4.6 $\pm$ 0.3	0.4169
Triacylglycerol (mmol/L)	1.4 $\pm$ 0.3	2.6 $\pm$ 0.4*	0.036
Aspartate transaminase (U/L)	18.3 $\pm$ 2.9	26.3 $\pm$ 2.4	0.1
Alanine transaminase (U/L)	18.3 $\pm$ 5.8	35.3 $\pm$ 2.9*	0.043

Anthropometric data. All patients undertook a 12-h fasting before blood collection for biochemical tests. Fasting was either self-administered or managed during the hospital stay. Clinical biochemistry testing was conducted by Hitachi Automatic Biochemistry Analyzer (Hitachi High-technologies Corporation, Tokyo). HbA1c was analyzed by high-performance liquid chromatography (Bio-Rad-D10). All values are expressed as mean $\pm$ S.E.M, $n=3$, * $P<0.05$ vs lean participants.

well of cultured cells. Lentiviral activation particles were thawed at room temperature and mixed gently before use. AMPK α 1 lentiviral activation particles or control lentiviral activation particles were administered to cultured STC-1 cells. Culture plates were swirled gently to mix the lentivirus. Then, the cells were incubated for 48 h.

Recruitment of human subjects and collection of colon mucosa

Three obese male participants with type 2 diabetes and 3 age-matched lean normal glycemic male participants were enrolled in the study. Anthropometric data are provided in Table 1 (ESM). Participation in this study was voluntary, and written informed consent was obtained from each participant. The guidelines of the Declaration of Helsinki (2000) of the World Medical Association were followed. All protocols were approved by the Research Ethics Committee of The First Affiliated Hospital of Jinan University.

All participants were fasted for 8 h. An enteroscopy was performed in sedated participants using a colonoscope (CF-HQ290I; Olympus). Mucosal biopsies were taken from colon. Tissue samples were extracted for protein and RNA using lysis buffer and RNA TRIzol, respectively.

Western blot analysis

Colon mucosa and culture cells were quickly harvested, rinsed thoroughly with PBS and then homogenized on ice in the protein lysis buffer (50 mmol/L Tris-HCl; 15 mmol/L EGTA; 100 mmol/L NaCl; 0.1% Triton X-100 supplemented with protease inhibitor cocktail, pH 7.5). After centrifugation for 10 min at 4°C, the supernatant was used for Western blot analysis. Protein concentration was measured by Bradford's method. Proteins were loaded onto SDS-PAGE gels and then transferred to nitrocellulose membranes. The membranes were incubated for 1 h at room temperature with 5% fat-free milk in Tris-buffered saline containing Tween 20, followed by incubation overnight at 4°C with the primary antibodies. Specific reaction was detected using IRDye-conjugated second antibody and visualized using the Odyssey Infrared Imaging System (LI-COR Biosciences, Lincoln, NE, USA).

RNA extraction and quantitative real-time PCR analysis

For gene expression analysis, RNA was isolated from colon mucosa and STC-1 cells using TRIzol (Invitrogen) and reverse transcribed into cDNAs using the First-Strand Synthesis System for RT-PCR kit (Invitrogen).

SYBR Green-based quantitative RT-PCR was performed using the Mx3000 multiplex quantitative PCR system (Stratagene). Triplicate samples were collected for each experimental condition to determine relative expression levels. Sequences for the primer pairs used in this study are as follows: mouse proglucagon (accession no. NM_008100.3), sense 5'-ATT GCC AAA CGT CAT GAT GA-3' and antisense 5'-GGC GAC TTC TTC TGG GAA GT-3'; mouse β -actin (accession no. NM_007393.3), sense 5'-ATC TGG CAC CAC ACC TTC-3' and antisense 5'-AGC CAG GTC CAG ACG CA-3'; human proglucagon (accession no. NM_002054.4), sense 5'-GCA CAT TCA CCA GTG ACT ACA GCA-3' and antisense 5'-TGG CAG CTT GGC CTT CCA AAT A-3'; human TFIIB (accession no. NM_001514.5), sense 5'-ACC AGC CGT TTG GAT GCT C-3' and antisense 5'-CCC ACA TCA ATA ACC CGG TC-3'.

Measurements of GLP1

Measurements of GLP1 secretion were performed as described previously (Xu *et al.*, 2015b). Samples were collected in the presence of aprotinin (2 μ g/mL), EDTA (1 mg/mL) and Diprotin (0.1 mmol/L). Cell culture medium was harvested and stored at -80°C before use. Glucagon-like peptide-1 (active forms) was assayed using the enzyme immunoassay kit according to the manufacturer's instruction, which is highly specific for the immunologic measurement of active GLP1(7-36 amide) and GLP1(7-37) in plasma and will not detect other forms of GLP1 (e.g., 1-36 amide, 1-37, 9-36 amide or 9-37).

Statistical analysis

All data were expressed as mean $\pm$ S.E.M. Statistical significance was analyzed with an unpaired Student's *t*-test. Data were considered significant when *P* < 0.05.

Results

Effects of glucose and serum deprivation on the phosphorylation of AMPK α and expression of GLP1 in STC-1 cells

As shown in Fig. 1A, phosphorylation of endogenous AMPK α on Thr172 in STC-1 cells was decreased by high glucose relative to low glucose. This change was associated with an increase in GLP1 synthesis and secretion. mRNA (Fig. 1B) and protein levels (Fig. 1A) of proglucagon and GLP1 secretion

(Fig. 1C) were significantly higher when STC-1 cells were cultured at 25 mmol/L glucose than at 5.6 mmol/L glucose.

The effects of serum deprivation on AMPK α phosphorylation and GLP1 production were next examined. Serum starvation significantly inhibited the proglucagon mRNA and protein, as well as GLP1 secretion, but stimulated AMPK α phosphorylation (Fig. 1D–F).

These *in vitro* experiments indicate that negative energy balance upregulates AMPK α phosphorylation, which subsequently inhibits GLP1 production in L-like

cells. We then examined the effects of long-term changes in nutritional status on AMPK α phosphorylation and GLP1 production in colon mucosa of human subjects. As shown in Fig. 2A, levels of phosphorylated AMPK α in colon mucosa were significantly decreased in obese patients with $41.2 \pm 7.8 \text{ kg/m}^2$ body mass index (BMI) relative to lean human subjects ($23.3 \pm 2.2 \text{ kg/m}^2$). In contrast to the changes in AMPK α , proglucagon mRNA (Fig. 2B) and protein (Fig. 2A) demonstrated a significant increase in obese subjects compared with lean people.

Figure 1

Effects of glucose and serum deprivation on the phosphorylation of AMPK α and expression of GLP1 in STC-1 cells. Cultured STC-1 cells were incubated for 24 h in the presence of 5.6 mmol/L (low) or 25 mmol/L (high) glucose supplemented with 2.5% fetal bovine serum and 10% horse serum (A–C). STC-1 cells were maintained in high glucose DMEM medium supplemented with indicated serum or serum deprivation for 24 h (D–F). Shown are representative Western blots for phospho-AMPK α (pAMPK α , Thr172) and proglucagon. AMPK α and β -actin were used as loading controls (A and D). Proglucagon mRNA (B and E) was analyzed by quantitative PCR, normalized to β -actin and expressed as fold change from control. Medium GLP1 (C and F) was determined by enzyme immunoassay. Results are expressed as mean $\pm$ s.e.m. Experiments were repeated for three times. *Denotes $P < 0.05$ vs control.

Inhibition of GLP1 synthesis and secretion by AMPK α 1 in STC-1 cells

The direct effects of AICAR, an activator of AMPK, on GLP1 synthesis and secretion were next examined in cultured STC-1 cells. Exposure of STC-1 cells to AICAR at the doses ranging from 31.25 to 250 μ mol/L for 24 h caused a concentration-dependent increase in AMPK α phosphorylation (Fig. 3A) and a decrease in proglucagon mRNA (Fig. 3B) and protein (Fig. 3A) and medium content of GLP1 (Fig. 3C).

Figure 2
Downregulation of colonic AMPK α and upregulation of GLP1 production in obese human subjects. (A) Representative Western blot from colon mucosa of lean and obese human subjects. Phospho-AMPK α (pAMPK α , Thr172) and proglucagon were blotted as described in 'Materials and methods' section. AMPK α and β -actin were used as loading controls. Quantification of image analysis of colonic AMPK α phosphorylation and proglucagon expression is expressed as mean $\pm$ S.E.M. (B) Results of quantitative PCR analysis of proglucagon mRNA are expressed as fold increase from lean subjects using TFII as loading control. Three colonic samples were examined for each condition. * P < 0.05 vs lean subjects.

Consistent with the pharmacological AMPK, overexpression of wild-type AMPK α 1 (WT) or constitutively active AMPK α 1 (AF) plasmids significantly inhibited the proglucagon mRNA and protein, as well as GLP1 secretion in cultured STC-1 cells (Fig. 4A–C). On the other hand, there is no significant change in GLP1 production in STC-1 cells overexpressing AMPK α 2 plasmids (Supplementary Fig. 1, see section on supplementary data given at the end of this article). Overexpression of constitutively active AMPK α 1 upregulated the expression and phosphorylation of AMPK α 1 (Fig. 4A). Further, overexpression of AMPK α 1 lentivirus in cultured STC-1 cells significantly enhanced the phosphorylation and expression of AMPK α 1 (Fig. 4D). The increment of AMPK α 1 was associated with a decrease of proglucagon mRNA and protein levels (Fig. 4E and D), as well as GLP1 secretion (Fig. 4F).

Stimulation of GLP1 synthesis and secretion by inhibition of AMPK α 1 in STC-1 cells

The direct effects of selective and reversible inhibition of AMPK on GLP1 synthesis and secretion were next examined by treating cultured STC-1 cells with Compound C. As shown in Fig. 5A–C, Compound C, at a dose ranging from 1.25 to 20 μ mol/L, induced a concentration-dependent increase in GLP1 synthesis and secretion. Compound C caused a concentration-dependent inhibition in AMPK α phosphorylation, which was associated with an increment in proglucagon mRNA and protein levels, as well as GLP1 release (Fig. 5A–C). Further, Compound C pretreatment abolished the AICAR-induced reduction in GLP1 synthesis and secretion, and enhancement of AMPK α phosphorylation (Fig. 5D–F).

Transfection of a kinase-inactive AMPK mutant (AMPK α 1-DN) plasmid caused a marked the GLP1 synthesis and secretion (Fig. 4A–C). Levels of phospho-AMPK α 1 were significantly decreased in cells transfected with AMPK α 1-DN.

On the other hand, siRNA knockdown of endogenous AMPK α 1 significantly increased proglucagon mRNA and protein levels (Fig. 6 A and B), as well as GLP1 secretion (Fig. 6C) in cultured STC-1 cells. Knockdown of AMPK α 1 markedly inhibited the phosphorylation and expression of AMPK α 1 (Fig. 6A).

Discussion

The major finding of this study is that AMPK in STC-1 cells functions to alter the expression and secretion of GLP1. This conclusion is supported by the following distinct

Figure 3

Suppression of GLP1 synthesis and secretion by AICAR in STC-1 cells. Cultured STC-1 cells were treated with different concentrations of AICAR for 24 h. Proglucagon mRNA (B) and protein (A) were analyzed by RT-PCR and Western blotting. Medium GLP1 (C) was determined by enzyme immunoassay. Results are expressed as mean $\pm$ S.E.M. Experiments were repeated for three times. * $P < 0.05$ vs control.

observations: (1) there exists a negative relation between AMPK α phosphorylation and the production of GLP1 in response to negative energy supply such as glucose deficiency and serum deprivation in cultured STC-1 cells or positive energy balance in obese human subjects; (2) stimulation of AMPK α 1 by either pharmacological or genetic approaches suppresses proglucagon mRNA

and protein content in STC-1 cells; and (3) conversely, inhibition of AMPK α 1 signaling by Compound C, AMPK α 1 siRNA or a dominant negative form of AMPK α 1 increases the expression and secretion of GLP1.

Enteroendocrine L cells are mainly expressed in the distal ileum and colon. In response to nutrients such as glucose and monounsaturated fatty acids, these

Figure 4

Regulation of GLP1 production by overexpression of AMPK α 1 gene in STC-1 cells. Effects of AMPK α 1 plasmids (A–C). STC-1 cells were transfected for 48 h with GFP, wild-type AMPK α 1 (WT), constitutively active form of AMPK α 1 (AF) or a dominant negative form of AMPK α 1 (DN) plasmids using Lipofectamine. Phospho-AMPK α , proglucagon, AMPK α 1 and β -actin (A), proglucagon mRNA (B), and medium GLP1 (Active) concentration (C) were measured and expressed as mean $\pm$ S.E.M. $n = 3$; * $P < 0.05$ vs control. Effects of AMPK α 1 lentivirus (D–F). STC-1 cells were infected with control lentivirus (CV) or lentiviral particles coding for AMPK α 1 (LV-AMPK α 1) for 48 h. (D) Representative Western blots of phospho-AMPK α , proglucagon, AMPK α 1 and β -actin. (E) Proglucagon mRNA. (F) Medium GLP1 (Active) concentration. Results were expressed as mean $\pm$ S.E.M. $n = 3$; * $P < 0.05$ vs control.

Figure 5

Stimulation of GLP1 synthesis and secretion by Compound C in STC-1 cells. (A–C) Cultured STC-1 cells were treated with varying concentrations of Compound C for 24 h. Phospho-AMPKα, AMPKα and proglucagon protein were analyzed by Western blotting (A). Results of quantitative PCR analysis of proglucagon mRNA are expressed as fold increase from vehicle control using β-actin as loading control (B). Medium GLP1 (C) was determined by enzyme immunoassay. Results are expressed as mean ± s.e.m. Experiments were repeated for three times. **P* < 0.05 vs control. (D–F) Cultured STC-1 cells were treated with DMSO, AICAR (125 μM), Compound C (10 μM) or AICAR plus Compound C for 24 h. Phospho-AMPKα, AMPKα and proglucagon protein (D) were analyzed by Western blotting. Proglucagon mRNA (E) was analyzed by RT-PCR. Medium GLP1 (F) was determined by enzyme immunoassay. Results are expressed as mean ± s.e.m. Experiments were repeated for 3 times. **P* < 0.05 vs control.

cells secrete GLP1 (Mansour *et al.* 2013). The molecular mechanism by which L cells sense nutrients to and then alter the secretion of GLP1 remains largely unknown. Adenosine 5'-monophosphate (AMP)-activated protein

kinase (AMPK), mammalian target of rapamycin (mTOR) and peroxisome proliferator-activated receptors (PPARs) have been reported to serve as crucial cellular energy sensors to regulate cell metabolism (Jiang *et al.* 2015).

Figure 6

Effects of AMPKα1 gene manipulation on GLP1 production. STC-1 cells were transfected with control siRNA or AMPKα1 siRNA for 48 h. (A) Representative Western blots of phospho-AMPKα, proglucagon, AMPKα1 and β-actin. (B) Proglucagon mRNA. (C) Medium GLP1 (Active) concentration. Results were expressed as mean ± s.e.m. *n* = 3; **P* < 0.05 vs control.

We thus speculate that GLP1 biosynthesis and secretion can be modulated by these nutrient-sensing molecules. Our previous report has demonstrated that mTORC1 may function to integrate nutritional and hormonal signals such as ghrelin with the synthesis and secretion of GLP1 within L cells (Xu *et al.* 2015a,b). This study extends the nutrient-sensing mechanism in L cells to AMPK signaling. Our finding demonstrates that AMPK α 1 is altered by cellular energy status in STC-1 cells, an L-like cell line, and in colon mucosa of human subjects. Phosphorylation of AMPK α 1 increases during negative energy balance such as glucose deficiency or serum deprivation, whereas its level decreases during long-term energy surplus in obese human subjects. These observations are consistent with other studies demonstrating that AMPK is critical for nutrient and hormone sensing in a variety of cells ranging from central nervous system to peripheral tissues (Kim & Lee 2005). In hypothalamic neurons, AMPK integrates nutritional signals with feeding behavior (Minokoshi *et al.* 2004, De Morentin *et al.* 2011, Stark *et al.* 2013). In muscle and liver, AMPK improves insulin sensitivity (Friedrichsen *et al.* 2013, Hasenour *et al.* 2013). In pancreatic islets, AMPK links the extracellular glucose with transcription of preproinsulin and secretion of insulin (Da Silva Xavier *et al.* 2000, 2003). Using pharmacological and genetic intervention for gain or loss of function of AMPK α 1, we demonstrate that AMPK α 1 negatively regulates production and secretion of GLP1 in L cells. By simultaneously inducing glucose-dependent stimulation of insulin secretion and suppressing glucagon secretion, GLP1 functions as a potent antihyperglycemic hormone (Baggio & Drucker 2007). Modulation of GLP1 synthesis and secretion by AMPK in L cells may thus coordinate the level of glucose in an organism with secretion of insulin, leading to the fine control of glucose homeostasis.

AMPK is a serine/threonine protein kinase that acts as a sensor of cellular energy homeostasis. It is expressed as a heterotrimer consisting of one catalytic α -subunit and 2 regulatory subunits (β and γ) (Stapleton *et al.* 1997). The α -subunit of AMPK is encoded either by the PRKAA1 or PRKAA2 gene, whereas the β - and γ -subunits are encoded by the PRKAB1 or PRKAB2 and the PRKAG1, PRKAG2 or PRKAG3 genes, respectively, yielding 12 possible heterotrimeric complexes, which provide a molecular basis for the multiple roles of the highly conserved AMPK signaling system in nutrient regulation and utilization in distinct mammalian cells (Stapleton *et al.* 1997, Gao *et al.* 1996). The different complexes of AMPK confer tissue specificity. Isoform-selective

activators or inhibitors of AMPK may thus provide new therapeutic tools for the treatment of type 2 diabetes mellitus. Intestinal AMPK also fulfills key functions in metabolic processes. The prevailing catalytic subunit of AMPK complex in small intestine is α 1 and its preferential associated regulatory partners are β 2 and γ 1 (Harmel *et al.* 2014). Because each isoform varies from its sibling forms, the complement of isoforms present in a complex can influence the role and response of AMPK within the cell. In muscle, AMPK α 2 is the predominant subunit representing two-thirds of the total α mRNA (Zong *et al.* 2002). Exercise increases nuclear translocation of AMPK α 2 (Goodyear & Kahn 1998, Birk & Wojtaszewski 2006), while in AMPK α 2 by overexpressing its domain-negative mutant decreased glucose uptake in skeletal muscle in response to contraction (Mu *et al.* 2001, Steinberg *et al.* 2006). In pancreatic β -cells, the α 2-subunit displays substantial nuclear localization and is thus implicated in the control of gene expression. On the other hand, α 1-containing complex whose total activity exceeds that of α 2-containing complex by 5- to 10-fold is mainly cytosolic and is proposed to regulate plasma membrane ion channels and secretion of insulin (da Silva Xavier *et al.* 2000, 2003). Previous studies have showed that AICAR, an AMPK activator, increases GLP1 secretion in rodents but not in fetal rat intestinal cells, murine GLUTag or human NCI-H716 L cells (Maida *et al.* 2011, Mulherin *et al.* 2011). Our findings suggest that AMPK α 1 alters both the transcription and secretion of GLP1 in STC-1 cells. AMPK α 1-containing complex suppresses proglucagon mRNA and protein and the secretion of GLP1 by overexpressing this subunit. Inhibition of AMPK α 1 signaling by AMPK α 1 siRNA or a dominant negative form of AMPK α 1 increases the expression and secretion of GLP1. Our experiments thus provide evidence that AMPK α 1 modulates GLP1 synthesis and release in the STC-1 cell line, a remarkable intestinal L cell model. These findings are consistent with our previous observations demonstrating that mTOR activity stimulates GLP1 production in L-like cells (Xu *et al.* 2015a,b). AMPK has been well characterized to suppress mTOR signaling through tuberous sclerosis complex 1 and 2 (TSC1/2) (Inoki *et al.* 2006). AMPK may thus inhibit GLP1 through mTOR in L cells.

As an insulinotropic and glucagonostatic hormone that inhibits appetite and reduces body weight, GLP1 is a promising therapy for type 2 diabetes (Baggio & Drucker 2007). GLP1 receptor agonists (Buse *et al.* 2004)

and dipeptidyl peptidase-4 inhibitors (Aschner *et al.* 2006) have been widely used as treatment targeting β -cell dysfunction in patients with type 2 diabetes. However, the use of these drugs may be associated with certain adverse effects on pancreatic and thyroid tissues. Both animal studies and analyses of drug databases have indicated an association of GLP1 receptor agonists with pancreatitis, pancreatic cancer and thyroid cancer (Bjerre *et al.* 2010, Parks & Rosebraugh 2010). Our study provides an alternative strategy for manipulation of GLP1 by altering AMPK activity in colon mucosa. If the relationship of AMPK signaling with GLP1 production exists in normal and diabetic humans, it might be feasible to target AMPK in colon mucosa for the therapy of type 2 diabetes. In summary, the data presented here demonstrate that AMPK α 1 may link energy supply with the production of GLP1 in L-like cells.

Supplementary data

This is linked to the online version of the paper at <http://dx.doi.org/10.1530/JME-16-0099>.

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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Author contribution statement

Xu G and Zhang W designed the research; Jiang S, Zhai H, Li D, Huang J, Zhang H and Li Z performed the research; Xu G and Jiang S analyzed the data; Xu G and Zhang W wrote and edited the paper. All authors contributed to the discussion and revised the article and all of them approved the final versions of the manuscript. Xu G and Zhang W are responsible for the integrity of the work as a whole.

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