RESEARCH

Stat3-mTOR signaling mediates the stimulation of GLP-1 production induced by IL-27

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Abstract

GLP-1 is a potent glucose-dependent insulinotropic hormone derived from intestinal L cells. Inflammatory Interleukin-27 (IL-27), a pleiotropic two-chain cytokine, is composed of EBI3 and IL-27 p28 subunits. IL-27 has a protective effect on pancreatic β -cell function. The relationship between IL-27 and GLP-1 is still unexplored. Here we showed interleukin-27-stimulated GLP-1 production via the Stat3-mTOR-dependent mechanism. Interleukin 27 receptor subunit alpha (IL-27 R α) was detected in ileum and STC-1 cells. Co-localization of EBI3 and GLP-1 was observed not only in mouse ileums but also in human ileums and colons. Third-ventricular infusion of IL-27 increased ileal and plasma GLP-1 in both lean C57BL/6J mice and diet-induced obese and diabetic mice. These changes were associated with a significant increase in Stat3-mTOR activity. Treatment of STC-1 cells with IL-27 contributed to the increments of Stat3-mTOR signaling and GLP-1. Interference of mTOR activity by mTOR siRNA or rapamycin abolished the stimulation of GLP-1 production induced by IL-27 in STC-1 cells. Stat3 siRNA also blocked the stimulus effect of IL-27 on GLP-1. IL-27 increased the interaction of mTOR and Stat3 in STC-1 cells. Our results identify Stat3-mTOR as a critical signaling pathway for the stimulation of GLP-1 induced by IL-27.

Key Words

- ▶ interleukin-27
- ▶ GLP-1
- ▶ mTOR
- ▶ Stat3

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Introduction

Glucagon-like peptide 1 (GLP-1) is an intestinal incretin produced in L cells through proglucagon processing (Holst 2007, Drucker *et al.* 2017). The majority of circulating biologically active GLP-1 is found in the GLP-1(7-36) amide form, with a lesser amount of the bioactive GLP-1(7-37) form also detectable (Kreymann *et al.* 1987). The biological activities of GLP-1 include stimulation of glucose-dependent insulin secretion and insulin biosynthesis, inhibition of glucagon secretion and gastric emptying (Andersen *et al.* 2018). Hence, identification of factors promoting endogenous GLP-1 release is of interest

because enhancing endogenous GLP-1 may be a useful strategy to prevent pancreatic β -cell failure in insulin-resistant obese patients and subsequent development of type 2 diabetes (Tuduri *et al.* 2016). Current incretin therapies include incretin mimetics (i.e. GLP-1 receptor agonists) and inhibitors of dipeptidyl peptidase IV (DPPIV or CD26) (incretin enhancers), an enzyme responsible for cleavage of GLP-1 to inert metabolites (Drucker & Nauck 2006). Incretin therapies offer great potential for the treatment of hyperglycemia without causing hypoglycemia (Rosenstock *et al.* 2008). Therefore,

targeting an increase in endogenous GLP-1 production through modulation of proglucagon gene transcription in enteroendocrine L cells provides an exciting new therapeutic opportunity for diabetes.

Interleukin-27 (IL-27) is a pleiotropic two-chain cytokine. It consists of two subunits, an IL-12 p40-related protein, Epstein-Barr virus-induced gene 3 (EBI3, also known as IL-27 β or IL-27B) and an IL-12 p35 (p35)-related polypeptide, p28 (also known as IL-27 α , IL-27A, IL-27p28 or IL-30) (Pflanz *et al.* 2002). EBI3 is secreted as a 34 kDa glycoprotein and the p28 subunit is a 24.5 kDa polypeptide. The two subunits bind non-covalently to form IL-27 (Pflanz *et al.* 2002, Meka *et al.* 2015). IL-27 shares structural similarities with IL-12, IL-23 and IL-35. It is a new member of the IL-12 family (Vignali *et al.* 2012). IL-27 acts through a heterodimer receptor consisting of IL-27 R α (WSX1) and gp130 chains (Pflanz *et al.* 2004). The binding of IL-27 to its receptor, IL-27 R α , activates cytoplasmic domain Box 1 motif-binding site for JAK1/2, which subsequently activates Stat1 and Stat3 pathways. IL-27 also signals through p38 mitogen-activated protein kinase (MAPK) pathway (Lucas *et al.* 2003, Guzzo *et al.* 2010, Pot *et al.* 2011, Wang *et al.* 2011, Blahoianu *et al.* 2014). IL-27 inhibits streptozotocin (STZ)-induced hyperglycemia and pancreatic islet inflammation in type 1 diabetic mice (Fujimoto *et al.* 2011). Toll-like receptor-induced secretion of IL-27 is defective in newly diagnosed type-2 diabetic subjects (Madhumitha *et al.* 2018). Although IL-27 has gained great attention in recent years because of its therapeutic potential in treating diabetes (Fujimoto *et al.* 2011, Meka *et al.* 2015, Madhumitha *et al.* 2018), the underlying mechanisms remain to be further explored.

Our previous studies found that intestinal mTOR regulates GLP-1 mRNA and protein content in mouse L cells (Xu *et al.* 2015a,b). In the present study, our results indicate that IL-27 increases GLP-1 synthesis and secretion through Stat3-mTOR signaling, thus expanding its interest as a potential target for the treatment of type 2 diabetes mellitus.

Materials and methods

Materials

Interleukin-27 was from Biolegend, Inc. (San Diego, CA, USA). IFN- γ was from Sino Biological Inc. (Beijing, China). Rapamycin was purchased from Sigma Chemical Co.. Rabbit anti-Phospho-Stat3 (Tyr705), anti-Phospho-mTOR (Ser2448), anti-Phospho-p70 S6 Kinase (Thr389),

anti-Phospho-S6 (Ser235/236), anti-mTOR, anti-p70 S6 Kinase, anti-S6 antibodies, mouse anti-Stat3, anti- β -actin antibodies, Stat3 siRNA, mTOR siRNA and control siRNA were purchased from Cell Signaling Technology. Mouse anti-GLP-1 and rabbit anti-EBI3 antibodies were from Abcam Inc.. TRIzol reagent and reverse transcription (RT) system were purchased from Promega Inc.. Horseradish peroxidase-conjugated, donkey anti-rabbit IgG and donkey anti-mouse IgG were purchased from Jackson ImmunoResearch. Immobilon western chemiluminescent HRP substrate was purchased from Millipore. Lipofectamine was from Invitrogen Inc. Goat anti-mouse fluorescein isothiocyanate-conjugated IgG and dylight 594 affipure donkey anti-rabbit IgG (1:100) were from EarthOx LLC (San Francisco, CA, USA). Anti-rabbit IgG (Cat. A7016) and protein A+G agarose (Cat. P2012) were purchased from Beyotime. Glucagon-like peptide-1 enzyme immunoassay kit was purchased from Millipore.

Animals

Sixteen-week-old male C57BL/6J mice were used in present study. Four-week-old mice were fed in standard plastic rodent cages and maintained at a regulated environment (24°C, 12h light, 12h dark cycle with lights on at 7:00h and off at 19:00h) in Jinan University with *ad libitum* access to a normal chow diet (control diet, D12450; Research Diets) or high-fat diet (60% fat, D12492; Research Diets) for 12 weeks. Animals used in this study were handled in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 8023, revised 1978). All animal experiments were approved by the Animal Care and Use Committee of Jinan University.

Human specimen

Participation in this study was voluntary and written informed consent was obtained from each participant. The guidelines of the Declaration of Helsinki of the World Medical Association were followed. All protocols were approved by the Research Ethics Committee of the First Affiliated Hospital of Jinan University. Mucosal biopsies were taken from intestines.

Intracerebroventricular (icv) microinjections

For the animal treatment, IL-27 or interferon- γ (IFN- γ) was dissolved in saline on the day of treatment and

microinjected into third ventricle 1h before the onset of the dark phase. After 12-h fasting, lean C57BL/6J mice received either IL-27 (100ng) or saline in a total volume of 2 μ L by slow infusion in mice. High-fat diet-induced obese and diabetic mice received saline, IL-27 (100ng), IFN- γ (500pg) or IL-27 plus IFN- γ in a total volume of 2 μ L. All mice were returned to their home cages with free access to a premeasured amount of chow and water. Twenty-four hours after injection, blood samples and tissues were harvested.

Chloral hydrate (500mg/kg) was intraperitoneal subcutaneous injected before intracerebroventricular microinjections. A 26-gauge stainless steel guide cannula (Plastics One, Roanoke, VA) projecting into the third cerebral ventricle was implanted into each mouse using flat-skull coordinates from bregma (anteroposterior, -0.825mm; mediolateral, 0mm; dorsoventral, -4.8mm) (Dalvi *et al.* 2012).

Cell culture and transfection

Intestinal secretin tumor cell line (STC-1) was purchased from ATCC. 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. Cells were plated at optimal densities and grown for 24h, then transfected with control siRNA, mTOR siRNA or Stat3 siRNA using Lipofectamine reagent according to the manufacturer's instruction. After 24-h transfection, the cells were then treated with saline, IL-27 (50nmol/L) or IFN- γ (5pg/mL) for another 24h.

Co-immunoprecipitation (Co-IP)

For co-immunoprecipitation, STC-1 cells treated with IL-27 (50nmol/L) or PBS for 24h were lysed with RIPA lysis buffer for 30min and centrifuged at 12,000g for 15min. Total proteins (500 μ g) were incubated with indicating primary antibodies such as Stat3 and mTOR. The mixture was gently rotated at 4°C overnight. Anti-rabbit IgG antibody served as a negative control. The immunocomplex was collected with protein A+G agarose, and the precipitates were washed five times with ice-cold PBS. Finally, proteins were released by boiling in sample buffer and utilized for Western blot analysis.

Immunohistochemistry

Intestinal mucosal biopsies were postfixed in 4% paraformaldehyde, dehydrated, embedded in wax, and sectioned at 6 μ m. Paraffin-embedded sections were

dewaxed, rehydrated and rinsed in PBS. After boiling for 10min in 0.01mol/L sodium citrate buffer (pH 6.0), sections were blocked in 5% goat preimmune serum in PBS for 1h at room temperature and then incubated overnight with rabbit anti-EBI3 (1:50) combined with mouse monoclonal antibody to GLP-1 (1:500). Tissue sections were then incubated at 22°C for 2h with a mixture of the following secondary antibodies: goat anti-mouse fluorescein isothiocyanate-conjugated IgG (1:50) and DyLight 594 affininpure donkey anti-rabbit IgG (1:100). Photomicrographs were taken under a confocal laser-scanning microscope (Leica).

Western blot analysis

Protein extracts were electrophoresed, blotted, and then incubated with primary antibodies. The antibodies were detected using 1:10,000 horseradish peroxidase-conjugated, donkey anti-rabbit IgG and donkey anti-mouse IgG. A Western blotting luminol reagent was used to visualize bands corresponding to each antibody. The band intensities were quantitated by Image J software.

RNA extraction, quantitative real-time PCR, and reverse transcription-PCR analysis

For gene expression analysis, RNA was isolated from mouse tissues or STC-1 cells using TRIzol and reverse-transcribed into cDNAs using the First-Strand Synthesis System for RT-PCR kit. SYBR green-based real-time PCR was performed using the Mx3000 multiplex quantitative PCR system (Stratagene, La Jolla, CA, USA). Triplicate samples were collected for each experimental condition to determine relative expression levels. Sequences for the primer pairs used in this study follow:

mouse IL-27 R α (accession no. NM_016671.3)
sense 5'-GCTCTGCCCTGGTTTCTGTC-3'
antisense 5'-CTCCTTGATGTAAGGTTGCCC-3'
mouse proglucagon (accession no. NM_008100.4)
sense 5'-ATTGCCAAACGTCATGATGA-3'
antisense 5'-GGCGACTTCTTCTGGGAAGT-3'
mouse β -actin (accession no. NM_007393.5)
sense 5'-CCACAGCTGAGAGGGAAATC-3'
antisense 5'-AAGGAAGGCTGGAAAAGAGC-3'

For reverse transcription-PCR, IL-27 receptor mRNA was amplified in 25 μ L volumes and the amplification parameters consisted of initial denaturation at 94°C for 5min, followed by 30 cycles of denaturation at 94°C for 1min, annealing at 58°C for 30s and extension at 72°C

for 1 min. The resulting products were visualized by electrophoresis on a 2% agarose gel.

Measurements of GLP-1

Measurements of GLP-1 secretion were performed as described previously (Xu *et al.* 2015a,b). Blood samples were collected after anesthesia in the presence of aprotinin (2 µg/mL), EDTA (1 mg/mL) and diprotin (0.1 mmol/L). Plasma and cell culture medium were harvested and stored at -80°C before use. Glucagon-Like Peptide-1 (Active) was assayed using the enzyme immunoassay kit according to the manufacturer's instruction, which is highly specific for the immunologic measurement of active GLP-1 (7–36 amide) and GLP-1 (7–37) in plasma and will not detect other forms of GLP-1 (e.g., 1–36 amide, 1–37, 9–36 amide, or 9–37).

Statistical analysis

All values are expressed as means ± s.e.m. Statistical differences were evaluated by two-way ANOVA and Newman–Student–Keuls test. Comparisons between two groups involved use of the Student's *t* test. *P* value <0.05 denotes statistical significance.

Results

Expression of IL-27 R α

IL-27 binds to and activates the IL-27 R α (WSX-1) and gp130 to exercise its physiological functions (Pflanz *et al.* 2004). To examine the expression of IL-27 R α , we analyzed the expression of IL-27 R α in different tissues and STC-1 cells, an intestinal secretin tumor cell line in which GLP-1 is abundantly expressed. As shown in Fig. 1, IL-27 R α mRNA was detected in hypothalamus, stomach, duodenum, jejunum, ileum, liver, pancreas and the muscle. IL-27 R α was also expressed in STC-1 cells.

Expression and co-localization of EBI3 and GLP-1 in the human and mouse intestinal mucosa

EBI3 (Epstein-Barr virus-induced gene 3) also known as IL-27 β or IL-27B is an IL-27-specific subunit (Pflanz *et al.* 2002). We examined the co-localization of EBI3 and GLP-1 in L cells using double-labeling immunofluorescent staining. Antibodies recognizing EBI3 and GLP-1 demonstrated strong positive reactivity in mouse ileums (Fig. 2A), human ileums (Fig. 2B), and human colons (Fig. 2C). Immunofluorescence staining showed virtually 100% of GLP-1-positive cells stained positively for EBI3,

Figure 1

Expression of IL-27 R α mRNA in tissues and STC-1 cells. Expression of IL-27 R α mRNA in tissues and STC-1 cells. Shown is the representative of three individual reverse transcription-PCR and real-time PCR. β -Actin was used as internal control.

and 40 ± 5% of EBI3-positive cells expressed GLP-1 in mouse ileums (Fig. 2A). Nearly all human ileal L cells stained positively for EBI3, and 80 ± 5% EBI3-positive cells expressed GLP-1 (Fig. 2B). In human colons, about 80% of GLP-1-positive cells stained positively for EBI3, while 70 ± 5% EBI3-positive cells expressed GLP-1 (Fig. 2C).

Effects of the third icv interleukin-27 on GLP-1 synthesis in both lean and diet-induced obese and diabetic mice

Short-term effects of IL-27 on GLP-1 were first examined in C57BL/6J mice fed with standard chow. We found that the third icv injection of IL-27 (100 ng/2 µL) significantly increased ileal GLP-1 protein (Fig. 3A) and mRNA (Fig. 3B) levels as well as circulating GLP-1 (Fig. 3C).

Our previous study suggests that mTOR integrates nutritional and hormonal signals to regulate the synthesis and secretion of GLP-1 (Xu *et al.* 2015a,b). Thus, we examined the effect of IL-27 on mTOR signaling. IL-27 significantly increased the phosphorylation of ileal mTOR and its downstream target molecules such as p70s6 ribosomal protein kinase (S6K1) and ribosome S6 protein (S6) (Fig. 3A), indicating the activation of mTOR signaling. The previous study has shown that IL-27 activates both Stat1 and Stat3 through distinct IL-27 receptor subunits such as WSX-1 (IL-27 R α) and gp130 (Lucas *et al.* 2003, Guzzo *et al.* 2010, Pot *et al.* 2011, Wang *et al.* 2011). IL-27 also stimulated the activity of Stat3 in the current study (Fig. 3A).

Similar effects of IL-27 on GLP-1 were also observed in high-fat diet-induced obese and diabetic mice. Central administration of IL-27 (100 ng/2 µL) significantly upregulated protein (Fig. 3D) and mRNA (Fig. 3E) levels of GLP-1 in the ileums as well as circulating GLP-1 (Fig. 3F) in diet-induced obese and diabetic mice.

Figure 2

Co-localization of EBI3 and GLP-1 in mouse and human. Images depicting EBI3 (red) and GLP-1 (green) in mouse ileums. The merged image illustrates the co-localization of EBI3 and GLP-1 (orange) (A). Bar, 25 μ m. Images depicting EBI3 (red) and GLP-1 (green) in the human ileums. The merged image illustrates the co-localization of EBI3 and GLP-1 (orange) (B). Expression and co-localization of EBI3 (red) and GLP-1 (green) in human colons. Merged image illustrates the co-localization of EBI3 and GLP-1 (orange) (C). Bar, 25 μ m.

Interferon- γ (IFN- γ), a pleiotropic cytokine produced principally by CD4 TH1 cells, CD8 T cells, and NK cells, is essential for both innate and adaptive immunity (McLaughlin *et al.* 2017). Exogenous IL-27 induces the activation of mTOR through JAK/PI3K pathway and inhibits IFN- γ -stimulated autophagy (Sharma *et al.* 2014). Contrast to IL-27, inhibitory effects of IFN- γ on ileal and circulating GLP-1 were observed in diet-induced obese and diabetic mice (Fig. 3D, E and F). Moreover, IL-27 reversed the inhibition of Stat3-mTOR and GLP-1 induced by IFN- γ (Fig. 3D, E and F).

Stimulation of GLP-1 synthesis and secretion by interleukin-27 in STC-1 cells

We next examined the effects of IL-27 on GLP-1 synthesis and secretion in STC-1 cells. Consistent with *in vivo* studies, treatment of IL-27 for 24 h caused a

dose-dependent stimulation of proglucagon protein (Fig. 4A) and mRNA (Fig. 4B) levels, as well as the activation of Stat3-mTOR signaling. IL-27 also induced the release of GLP-1 (Fig. 4C).

IL-27 at 50 nmol/L significantly activated Stat3-mTOR pathway and upregulated proglucagon mRNA and protein levels, as well as GLP-1 secretion in a time-dependent manner in STC-1 cells (Fig. 4D, E and F).

Effects of interferon- γ (IFN- γ) on Stat3-mTOR signaling and GLP-1 expression in STC-1 cells

We then examined the effects of IFN- γ on GLP-1 production in STC-1 cells. Exposure of STC-1 cells to IFN- γ at the doses of 0.625–10 pg/mL for 24 h caused a concentration-dependent inhibition in the phosphorylation of Stat3, mTOR, S6K, and S6, which was associated with a decrease in GLP-1 expression (Fig. 5A and B) and secretion (Fig. 5C).

Figure 3

Effects of central IL-27 on ileal GLP-1 in C57BL/6 mice and diet-induced obese and diabetic mice. Representative western blots from lean male C57BL/6J mice that received the 3rd icv of saline (2 μ L) or IL-27 (100 ng/2 μ L) (A). pStat3, Stat3, pmTOR, mTOR, pS6K, S6K, pS6, S6, proglucagon, and β -actin were detected using specific antibodies. mRNA levels of proglucagon were measured using quantitative PCR analysis (B). Plasma GLP-1 was detected by ELISA (C). Six mice were examined for each condition. * P < 0.05 vs saline treatment. Representative Western blots from diet-induced obese and diabetic mice that received the third icv interleukin-27 (100 ng/2 μ L) or IFN- γ (500 pg/2 μ L) (D). pStat3, Stat3, pmTOR, mTOR, pS6K, S6K, pS6, S6, proglucagon, and β -actin were detected using specific antibodies. mRNA levels of proglucagon were measured using quantitative PCR analysis (E). Plasma GLP-1 was detected by ELISA (F). Six mice were examined for each condition. * P < 0.05 vs saline treatment. # P < 0.05 vs IFN- γ treatment.

IFN- γ at 5 pg/mL significantly decreased proglucagon mRNA and protein levels, and GLP-1 secretion in a time-dependent manner in STC-1 cells (Fig. 5D, E and F).

IL-27 reverses the reduction of GLP-1 induced by IFN- γ

Pretreatment of STC-1 cells with IL-27 reversed the decrease in proglucagon protein (Fig. 6A) and mRNA contents (Fig. 6B), as well as GLP-1 secretion (Fig. 6C) induced by IFN- γ .

Rapamycin blocks the increment of GLP-1 induced by IL-27

Our previous study demonstrates intestinal mTOR stimulates GLP-1 synthesis (Xu *et al.* 2015b). We thus

examined whether mTOR mediates the effects of IL-27 on GLP-1 production in STC-1 cells. Inhibition of mTOR activity by rapamycin blocked the increment of GLP-1 induced by IL-27 (Fig. 7A, B and C).

Modulation of GLP-1 synthesis by interleukin-27 through Stat3-mTOR signaling

We examined whether Stat3-mTOR mediates the effects of IL-27 on GLP-1 production. siRNA knockdown of mTOR abolished the IL-27-induced enhancement of proglucagon protein (Fig. 8A) and mRNA (Fig. 8B) levels, as well as GLP-1 secretion (Fig. 8C) in cultured STC-1 cells. Knockdown of mTOR significantly inhibited the phosphorylation of mTOR, S6K, and S6. Furthermore, siRNA knockdown of mTOR abolished IL-27-induced phosphorylation of Stat3.

Figure 4

IL-27 increases GLP-1 synthesis and secretion in STC-1 cells. Cultured STC-1 cells were treated with varying concentrations of Interleukin-27 (IL-27) for 24 h or IL-27 (50 nmol/L) for the time indicated. Proglucagon protein (A and D) and mRNA (B and E) were analyzed by Western blotting and real-time PCR. Medium GLP-1 (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. Concentration-dependent effects (A, B and C). Duration-dependent effects (D, E and F).

Stat3 siRNA suppressed GLP-1 production induced by IL-27, which was associated with the inhibition of mTOR activity (Fig. 8D, E and F).

Co-IP was further employed to investigate whether Stat3 and mTOR interacted with each other. IgGs against Stat3 and mTOR co-immunoprecipitated with each other, indicating an interaction between mTOR and Stat3. IL-27 treatment enhanced the interaction between Stat3 and mTOR (Fig. 8G and H).

Discussion

The major finding of the present study is that IL-27 stimulates the synthesis and secretion of GLP-1 through Stat3-mTOR signaling (Fig. 9). This conclusion is supported by the following distinct observations: (1) IL-27 $R\alpha$ is expressed in mouse hypothalamus, ileum and STC-1 cells; (2) co-localization of EBI3 and GLP-1 is observed not only

in mouse ileums but also in human ileums and colons; (3) intracerebroventricular interleukin-27 activates ileal Stat3-mTOR signaling, while simultaneously increases proglucagon mRNA and protein contents, as well as circulating GLP-1 in C57BL/6J mice fed either normal chow or high-fat diet; (4) interleukin-27 stimulates Stat3-mTOR activity and GLP-1 production in STC-1 cells; (5) conversely, IFN- γ inhibits the synthesis and secretion of GLP-1. Moreover, IL-27 reverses the inhibition of GLP-1 caused by IFN- γ ; (6) pharmacological and genetic interference of mTOR signaling blocks the up-regulation of GLP-1 induced by Interleukin-27 in STC-1 cells; (7) stat3 siRNA abolishes the stimulation of GLP-1 production induced by interleukin-27; (8) interleukin-27 increases the interaction between Stat3 and mTOR.

Interleukin cytokine family plays an important role in diabetes, especially IL-6 and IL-12 cytokine families. Compared with control subjects, serum IL-23

Figure 5

Regulation of Stat3-mTOR signaling and GLP-1 expression by IFN- γ in STC-1 cells. Cultured STC-1 cells were treated with various concentrations of IFN- γ for 24 h or IFN- γ (5 pg/mL) for the time indicated. Proglucagon protein (A and D) and mRNA (B and E) were analyzed by Western blotting and real-time PCR. Medium GLP-1 (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. Dose-dependent effects (A, B and C). Time-dependent effects (D, E and F).

(Roohi *et al.* 2014) was decreased, while IL-6 (Ma *et al.* 2011) and IL-12 (Wegner *et al.* 2008) were increased in patients with type 2 diabetes. Interleukin-27, a heterodimeric cytokine composed of the subunits p28 and EBI3, relates to both IL-6 and IL-12 cytokine families (Vignali *et al.* 2012). Previous reports have indicated that IL-27 is significantly decreased in proliferative diabetic retinopathy patients and newly diagnosed type-2 diabetic subjects (Madhumitha *et al.* 2018, Yan *et al.* 2018). After treatment with streptozotocin (STZ) for induction of diabetes, mice deficient in IL-27 subunit (EBI3^{-/-}) or IL-27 receptor subunit (WSX-1^{-/-}) showed worsening glucose tolerance and increased immune cell infiltration into the islets compared with control mice. Furthermore, intraperitoneal injection of IL-27 daily at a dose of 400 ng/day for 10 days significantly lowered non-fasting blood glucose levels in EBI3 gene-null mice (Fujimoto *et al.* 2011). Another study further illustrated that interleukin-6

enhances insulin secretion by increasing glucagon-like peptide-1 secretion from L cells and alpha cells (Ellingsgaard *et al.* 2011). Here we report that IL-27 exerts its stimulus function on GLP-1. IL-27 receptor is observed in ileum and STC-1 cells. Co-localization of EBI3 and GLP-1 exists not only in mouse ileums but also in human ileums and colons. Third ventricle administration of IL-27 increases the synthesis and secretion of GLP-1 in lean and diet-induced obese and diabetic mice. Our previous study has shown that mTOR, as a key sensor in GLP-1 production cells, positively regulates GLP-1 synthesis and secretion (Xu *et al.* 2015b). In the current study, IL-27 significantly stimulates mTOR signaling in both mouse ileums and STC-1 cells. The previous study has shown that IL-27 induces Stat1 and Stat3 phosphorylation and activation in human and murine cell lines and primary human T cells (Lucas *et al.* 2003, Guzzo *et al.* 2010, Pot *et al.* 2011, Wang *et al.* 2011). IL-27 also stimulated the

Figure 6

IL-27 reverses the inhibition of GLP-1 caused by IFN- γ in STC-1 cells. STC-1 cells were treated for 24 h with IL-27 (50 nmol/L) or IFN- γ (5 pg/mL) alone or combined with IL-27. pStat3, Stat3, pmTOR, mTOR, pS6K, S6K, pS6, S6, proglucagon, and β -actin (A), proglucagon mRNA (B), and medium GLP-1 concentration (C) were measured and expressed as mean $\pm$ s.e.m. Experiments were repeated for three times. * $P < 0.05$ vs control. # $P < 0.05$ vs IFN- γ treatment.

ileal and cellular Stat3 phosphorylation in our study. Interference of Stat3 or mTOR attenuated the transcription and translation of GLP-1 induced by IL-27. Moreover, IL-27 increased the interaction between Stat3 and mTOR. We thus speculate that IL-27 stimulates GLP-1 production through Stat3-mTOR signaling.

Previous studies have demonstrated that GLP-1 is an incretin hormone secreted from intestinal L cells in response to nutrient intake and acts on beta cells to induce insulin secretion in a glucose-dependent manner

(Mojsov *et al.* 1987, Fehmann & Habener 1992). Current incretin therapies include incretin mimetics and incretin enhancers (Drucker & Nauck 2006). By simultaneously inducing glucose-dependent stimulation of insulin secretion and suppressing glucagon secretion, GLP-1 functions as a potent antihyperglycemic hormone (Andersen *et al.* 2018). Enhancement of endogenous GLP-1 may be a novel and more physiological option in incretin-based therapy for diabetes. In the current study, IL-27 stimulates GLP-1 synthesis and secretion not only in

Figure 7

Rapamycin blocks the increment of GLP-1 induced by IL-27. STC-1 cells were treated for 24 h with IL-27 (50 nmol/L) or rapamycin (20 nmol/L) alone or combined with IL-27. pStat3, Stat3, pmTOR, mTOR, pS6K, S6K, pS6, S6, proglucagon, and β -actin (A), proglucagon mRNA (B), and medium GLP-1 concentration (C) were measured and expressed as mean $\pm$ s.e.m. Experiments were repeated for three times. * $P < 0.05$ vs control. # $P < 0.05$ vs IL-27 treatment.

Figure 8

Modulation of GLP-1 production by IL-27 through Stat3-mTOR signaling. STC-1 cells were transfected with control siRNA or mTOR siRNA and treated with IL-27 (50 nmol/L) or IFN-γ (5 pg/mL) for 24 h. Results were expressed as mean ± s.e.m. Experiments were repeated for three times. * $P < 0.05$ vs control. Representative Western blots of pStat3, Stat3, pmTOR, mTOR, pS6K, S6K, pS6, S6, proglucagon, and β-actin (A). Proglucagon mRNA (B). Medium GLP-1 concentration (C). STC-1 cells were transfected with control siRNA or Stat3 siRNA and treated with IL-27 (50 nmol/L) or IFN-γ (5 pg/mL) for 24 h. Results were expressed as mean ± s.e.m. Experiments were repeated for three times. * $P < 0.05$ vs control. Representative Western blots of pStat3, Stat3, pmTOR, mTOR, pS6K, S6K, pS6, S6, proglucagon, and β-actin (D). Proglucagon mRNA (E). Medium GLP-1 concentration (F). Immunoprecipitations/immunoblot assaying for interaction between endogenous Stat3 and mTOR (G and H). STC-1 cells were treated with IL-27 (50 nmol/L) for 24 h. The interaction between Stat3 and mTOR was detected by co-immunoprecipitation. Stat3 was precipitated using anti-Stat3 antibodies and co-precipitated mTOR were immunoblotted (G). mTOR was precipitated using anti-mTOR antibodies and co-precipitated Stat3 were immunoblotted (H). Experiments were repeated for three times.

control mice but also in diet-induced obese and diabetic mice; thus, we speculate that IL-27 represents a potential therapeutic candidate to improve type 2 diabetes by enhancing endogenous GLP-1.

GLP-1-expressing neurons are found in the enteric nervous system but also in brain regions such as the

nucleus tractus solitarius and the ventrolateral medulla (Lim *et al.* 2009). GLP-1 receptors have also been detected in vagal and dorsal root ganglia and the area postrema and hypothalamus in the central nervous system (Richards *et al.* 2014), indicating that the action of GLP-1 on gut function may be centrally or peripherally

Figure 9

Summary of putative mechanisms linking Stat3-mTOR signaling pathway and GLP-1 production elicited by IL-27 in L cells. IL-27 stimulates the activity of Stat3-mTOR signaling. IL-27 also enhances the interaction between Stat3 and mTOR, leading to the subsequent stimulation of GLP-1 production in L cells. A full colour version of this figure is available at <https://doi.org/10.1530/JME-19-0124>.

orchestrated. In current study, IL-27 $R\alpha$ is expressed in both hypothalamus and gastrointestinal tract. Thus, we speculate that exogenous IL-27 modulates GLP-1 production via central and peripheral effects. Limitations exist for our approaches that seek to assess the role of IL-27 in the control of GLP-1 production. Functional data from intraperitoneal injection of IL-27 and vagotomy will address this potential.

IL-27 is reported to promote intestinal epithelial barrier integrity (Diegelmann *et al.* 2012). IL-27 has established roles in inflammatory bowel disease (IBD) (Andrews *et al.* 2016). GLP-1 is also regarded as a novel and potent therapeutic tool against intestinal inflammation and diarrhea associated with IBD (Duan *et al.* 2017). In our study, we found that IL-27 can promote intestinal GLP-1 synthesis and secretion in C57BL/6J mice fed either normal chow or high-fat diet by activating Stat3-mTOR signaling. IL-27 may serve as an effective therapy for IBD by increasing the activity of endogenous GLP-1, indicating a novel cross-talk between the metabolic and immune systems.

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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Ethical approval

The biopsy specimens were obtained under protocols approved by the Research Ethics Committee of the First Affiliated Hospital of Jinan University and informed consent was obtained from all patients. All animal

experiments were undertaken with approval from the Laboratory Animal Ethics Committee of Jinan University.

Author contribution statement

Geyang Xu designed the research; Guoqing Lei, Linxi Chen, Miao Peng, Bolin Zeng, Qiaoxi Yang and Hening Zhai performed the research; Guoqing Lei, Linxi Chen and Geyang Xu analyzed data; Geyang Xu wrote and edited the paper. All authors contributed to the discussion and revised the article and all approved the final versions of the manuscript. Geyang Xu is responsible for the integrity of the work as a whole.

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