Atorvastatin Targets the Islet Mevalonate Pathway to Dysregulate mTOR Signaling and Reduce β-Cell Functional Mass

Linyan Shen,1 Yanyun Gu,2 Yixuan Qiu,2 Tingting Cheng,1 Aifang Nie,2 Canqi Cui,2 Chenyang Fu,2 Tingting Li,2 Xuexin Li,2 Lihong Fu,2 Yanqiu Wang,2 Qicheng Ni,2 Qidi Wang,2 Weiqing Wang,2 and Bo Feng1

Statins are cholesterol-lowering agents that increase the incidence of diabetes and impair glucose tolerance via their detrimental effects on nonhepatic tissues, such as pancreatic islets, but the underlying mechanism has not been determined. In atorvastatin (ator)-treated high-fat diet–fed mice, we found reduced pancreatic β-cell size and β-cell mass, fewer mature insulin granules, and reduced insulin secretion and glucose tolerance. Transcriptome profiling of primary pancreatic islets showed that ator inhibited the expression of pancreatic transcription factor, mechanistic target of rapamycin (mTOR) signaling, and small G protein (sGP) genes. Supplementation of the mevalonate pathway intermediate geranylgeranyl pyrophosphate (GGPP), which is produced by 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase, significantly restored the attenuated mTOR activity, v-maf musculoaponeurotic fibrosarcoma oncogene homolog A (MafA) expression, and β-cell function after ator, lovastatin, rosuvastatin, and fluvastatin treatment; this effect was potentially mediated by sGP prenylation. Rab5a, the sGP in pancreatic islets most affected by ator treatment, was found to positively regulate mTOR signaling and β-cell function. Rab5a knockdown mimicked the effect of ator treatment on β-cells. Thus, ator impairs β-cell function by regulating sGPs, for example, Rab5a, which subsequently attenuates islet mTOR signaling and reduces functional β-cell mass. GGPP supplementation could constitute a new approach for preventing statin-induced hyperglycemia.

Statins, 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase inhibitors, are widely prescribed to reduce blood cholesterol levels and have been indicated to reduce atherosclerotic cardiovascular risks (1). However, increasing evidence based on longitudinal cohorts with large populations indicates that statin therapy can increase the risk of new-onset type 2 diabetes in a dose-dependent manner (2–6). Previous studies have demonstrated that increased hepatic glycogenesis (7), delayed glucose clearance (8), mitochondrial dysfunction in islets (9), reduced butyrate production by the gut microbiota (10), reduced adipocyte browning (11), and adipocyte inflammation (12) underlie the diabetogenic effect of statins, which has caused concern about the use of statins in clinical practice.

Statin-targeted HMG-CoA reductase controls not only de novo cholesterol synthesis but also production of coenzyme Q10, heme, and isoprenoid intermediates, such as farnesyl pyrophosphate (FPP) and geranylgeranyl pyrophosphate (GGPP) (13). The main function of FPP and GGPP is to regulate small G protein (sGP) farnesylation and geranylgeranylation, respectively; thus, FPP and GGPP modulate sGP function. The sGP family is involved in a wide range of cellular processes (14,15), such as cytoskeletal remodeling, vesicle fusion, and secretory granule transportation, which also occur in pancreatic islets (16–18). β-Cell–specific ablation of GGPP synthase (GGPPS) results in β-cell dysfunction mediated by the deactivation of Rab27a, which is a Rab protein family member and sGP subtype (19). Thus, it is possible that...
statins might affect β-cell function by disturbing protein prenylation, but this hypothesis has not yet been tested.

Interestingly, some sGPs, such as Ras homolog enriched in brain (Rheb), serve as key upstream signals of mechanistic target of rapamycin (mTOR) signaling (20). mTOR is a key intracellular nutrient and growth factor sensor and is crucial for maintaining mature β-cell functional mass (21–26). Two distinct protein complexes, mTOR complex 1 (mTORC1) and mTORC2 (26), execute the downstream functions of mTOR. A growing body of evidence has shown that mTOR can also be targeted by statins (27–30). Thus, it is of interest to study whether the FPP, GGPP/mTOR axis mediates the effect of statins on islet function impairment and eventually induction of diabetes.

To address this question, we used both in vivo and in vitro approaches to characterize β-cell defects and investigate transcriptome changes in mouse islets after long-term exposure to atorvastatin (ator). Our results confirm that the GGPP/sGP/mTOR axis might be responsible for statin-induced β-cell defects. We also identified an sGP, Rab5a, which has not previously been reported to be involved in β-cell regulation, as a potential target for the treatment of statin-induced diabetes.

RESEARCH DESIGN AND METHODS

Reagents
Ator was provided as a courtesy by Pfizer, Inc. (New York, NY). Lovastatin, rosuvastatin, fluvasatin, FPP, GGPP, cholesterol, GTGT-298, and methyl-β-cyclodextrin (MβCD) were purchased from Sigma-Aldrich (St. Louis, MO).

Cell Culture
MIN6 cells were used and maintained in DMEM containing 25 mM/L glucose supplemented with 15% FBS, 5 μL/L β-mercaptoethanol, 2 mM/L l-glutamine, 100 IU/mL penicillin, and 100 μg/mL streptomycin at 37°C in 5% CO₂.

Animals
C57BL/6N mice were purchased from the Model Animal Research Center of Nanjing University (Nanjing, China). Eight-week-old male C57BL/6N mice were divided randomly into two groups: 1) a high-fat diet (HFD) group (n = 23), in which mice were fed an HFD that was 45% lipids (12451; Research Diets, New Brunswick, NJ) for 4 months, and 2) an HFD+ator group (n = 22), in which mice were fed an HFD containing 10 mg/kg/day ator according to previous studies in mice (31,32). All mice were housed under a 12-h light/12-h dark cycle and a 23 ± 1°C ambient temperature with free access to water. All procedures were approved by the Animal Use and Care Committee of Shanghai Jiao Tong University.

Metabolic Studies
Body composition was measured by an animal whole-body composition analyzer (100H; EchoMRI, Houston, TX). For the intraperitoneal glucose tolerance test, mice were fasted overnight for 16 h before being injected intraperitoneally with glucose (2 mg/kg body weight). Blood glucose was tested using tail blood samples at 0, 15, 30, 60, and 120 min after glucose injection. Fasting plasma triglyceride (TG) and total cholesterol (TC) levels were measured enzymatically in accordance with the manufacturer’s guidance (Kehua, Shanghai, China). Fasting plasma insulin was measured with an Ultrasensitive ALPCO Insulin ELISA Kit (ALPCO, Salem, NH).

Isolation, Culture, and Dissociation of Mouse Pancreatic Islets
Mouse islets were isolated as previously described (22). In brief, the islets were isolated using collagenase P digestion (Roche Applied Science, Mannheim, Germany). Ex vivo, primary islets were cultured with RPMI 1640 culture medium supplemented with 10% FBS.

Glucose-Induced Insulin Secretion Assay
MIN6 cells were incubated in low-glucose (3.3 mM/L) and fatty acid–free BSA Krebs-Ringer HEPES buffer (KRB; 120 mM/L NaCl, 4.8 mM/L KCl, 2.5 mM/L CaCl₂, 1.2 mM/L MgSO₄, 1.2 mM/L KH₂PO₄, 10 mM/L HEPES, 25 mM/L NaHCO₃) for 4 h before glucose-induced insulin secretion (GIIS) testing. Then, the cells were incubated with low glucose (3.3 mM/L) and fatty acid–free BSA KRB or with high-glucose (16.7 mM/L or 33.3 mM/L) KRB buffer at 37°C for 1 h. Then, the buffer supernatant was collected after 1 h of high-glucose stimulation for subsequent insulin analysis with an ELISA Kit (ALPCO) according to the manufacturer’s instructions.

Western Blotting and Membrane Fractionation
Proteins were assayed using a Pierce BCA Protein Assay Kit (Thermo Fisher Scientific, Waltham, MA). A Mem-PER Plus Kit (Thermo Scientific) was used to isolate membrane proteins and cytosolic proteins from 5.5 × 10⁶ MIN6 cells according to the manufacturer’s instructions. The antibodies used were as follows: anti–phospho-Akt Ser473, anti–phospho-S6 (Ser240/244), anti-raptor, anti–Na⁺/K⁺-ATPase (Cell Signaling Technology, Beverly, MA), anti-Hsp90 (Santa Cruz Biotechnology, Dallas, TX), anti–Rheb, anti–Rabggtb (Abcam, Cambridge, U.K.), horseradish peroxidase–conjugated anti–Gapdh (Kangcheng, Shanghai, China), anti–v-maf musculoaponeurotic fibrosarcoma oncogene homolog A (anti–MafA), anti–rictor (Bethyl Laboratories, Montgomery, TX), and anti–Rab5a (Proteintech, Chicago, IL).

β-Cell Mass Measurement and Immunostaining
The whole pancreas was removed, weighed, fixed, embedded in paraffin, and sectioned at a 5-μm thickness. Sections taken 250–300 μm apart were selected for insulin staining with a DAB Peroxidase Substrate Kit (Fuzhou Maixin Biotech, Fuzhou, China) and eosin counterstaining. The digital images of pancreatic sections were obtained using a Nikon MZ 100 microscope. The total pancreatic and insulin-positive areas of each section
were then measured with MetaMorph v6.1 (Molecular Devices), from which the β-cell mass was then calculated, as previously stated (22). Anti-Rab5a (1:200; Proteintech) and anti-Rheb (1:200; Abcam) antibodies were used for immunohistochemistry, and nuclei were stained with hematoxylin. For immunofluorescence, sections of pancreas were stained with anti-insulin (1:800; DAKO), anti-glucagon (1:500; Abcam), anti-MafA (1:1,000; Bethyl Laboratories), anti-pS6 (Ser240/244) (1:200; Cell Signaling Technology), anti-Ki67 (1:400; DSHB), and anti-Glut2 (1:400; Millipore) antibodies. Detection was performed using Alexa Fluor 488-, 594-, and 647-conjugated antibodies (Jackson ImmunoResearch or Life Technologies). Nuclei were counterstained with DAPI. Immunofluorescence images were acquired using an Olympus Microscope (Olympus, Tokyo, Japan) or Leica SP8 confocal microscope (Zeiss, Oberkochen, Germany).

Gene Expression Analysis
Total RNA was extracted with TRIzol (Invitrogen, Carlsbad, CA). cDNA was generated by Superscript II enzyme (Invitrogen) with random primers and then analyzed by real-time quantitative PCR with SYBR Green (Takara, Otsu, Shiga, Japan) on a LightCycler 480 (Applied Biosystems). The data were analyzed in SPSS 23.0 (SPSS Inc., Chicago, IL). An unpaired, two-tailed Student t test and a one-way ANOVA were used to analyze differences between two groups and among multiple groups, respectively. All results are expressed as the mean ± SEM. A value of P < 0.05 was considered to be significant. All experiments were repeated at least three times.

RNA Sequencing
Total RNA was extracted using an RNeasy Micro Kit (Qiagen, Venlo, the Netherlands). The sequencing libraries were generated using a VAHTS mRNA-seq v2 Library Prep Kit for Illumina (NR601; Vazyme) according to the manufacturer’s recommendations. The library concentration was measured using a Qubit RNA Assay Kit in Qubit 3.0 for preliminary quantification. The clustering of the index-coded samples was performed on a cBot Cluster Generation System (Illumina) according to the manufacturer’s instructions. After cluster generation, the prepared libraries were sequenced on the Illumina HiSeq X Ten platform.

Filipin Staining and Cholesterol Quantification
Filipin (Sigma-Aldrich) staining was used to visualize intracellular cholesterol. Cells were treated with ator (10 μmol/L), MβCD (1 mg/mL) (33), or vehicle for 24 h. Then, the cells were washed with PBS and fixed for 15 min in 4% formaldehyde. After permeabilization with 0.2% Triton, the cells were incubated with 50 μg/ml filipin in PBS for 45 min (34). A Total Cholesterol and Cholesterol Ester Colorimetric/Fluorometric Assay Kit (Biovision, Milpitas, CA) was used to quantify cholesterol levels from cell lysates according to the manufacturer’s instructions.

siRNA Transfections
Rab5a siRNA oligos siRab5a-mus-749 (CAGCCAUAGGUUGUAUGATT; UCAUACACAACUAUGGCGUGTT) and siRabggtb-mus-1122 (GGUGCUUCAGGGUGAAUTT; AUUACCUCUGAGGCACCTT) and control oligos were purchased from GenePharma (Suzhou, China). Lipofectamine 3000 transfection reagent was used (Invitrogen) according to the manufacturer’s instructions. Cells were collected after 72 h of transfection for further molecular studies.

Cell Cycle and Size Analyses
Flow cytometry was performed to investigate the cell cycle in the two groups (Cytofix5; Beckman Coulter, Brea, CA). After treatment for 24 h, cells were trypsinized, collected, and fixed with precooled 100% ethanol overnight at 4°C. Propidium iodide (BD Biosciences, New York, NY) was used to stain the cells for the cell cycle analysis. Cell size was determined by forward scatter (FSC) parameters (35). The data were analyzed by FlowJo v10 software.

Transmission Electron Microscopy
The pancreas was fixed, sectioned, and imaged as previously described (23). The diameter and subtype of insulin granules were determined by counting images captured at ×4,200 magnification. Representative images were captured at ×13,500 magnification.

Statistical Analyses
The data were analyzed in SPSS 23.0 (SPSS Inc., Chicago, IL). An unpaired, two-tailed Student t test and a one-way ANOVA were used to analyze differences between two groups and among multiple groups, respectively. All results are expressed as the mean ± SEM. A value of P < 0.05 was considered to be significant. All experiments were repeated at least three times.

Data and Resource Availability
Data supporting the findings of this work are available within the article and its Supplementary Data. The sequencing data for the RNA-seq analysis are available in the National Center for Biotechnology Information Sequence Read Archive (SRA) database under project PRJNA551568. All data supporting the findings of this study are available from the corresponding author on request.

RESULTS
Ator Treatment Impaired In Vivo Insulin Secretion
After 4 months of treatment (Fig. 1A), HFD mice treated with ator showed lower plasma TC (HFD vs. HFD+ator, 3.33 ± 0.08 vs. 2.82 ± 0.14 mmol/L; P = 0.04) (Fig. 1B) and TG levels (Fig. 1C) but higher fasting blood glucose levels and worse glucose tolerance (Fig. 1D–F) than untreated mice. There were no significant changes in body weight, body composition, or food intake (Supplementary Fig. 1A–D) after ator treatment. Furthermore, ator treatment significantly reduced the fasting insulin level (Supplementary Fig. 1E) and GIIS (Fig. 1G), whereas it slightly
increased peripheral insulin resistance (Supplementary Fig. 1F–H). These results suggested that insulin sensitivity and insulin secretion might both be affected by ator treatment.

Ator Impaired β-Cell Mass and Insulin Granule Maturation

We next sought to examine the ator-induced changes to the mouse endocrine pancreas. Ator treatment significantly reduced the β-cell mass (Fig. 2A and B) and β-cell size (Fig. 2C and D) in islets, as measured by the cell area surrounded by Glut2 immunolabeling. However, no changes in cell proliferation, according to the Ki67-positive β-cell percentage, were observed (Fig. 2E and Supplementary Fig. 2A). The islet structure in ator-treated mice assessed by the ratio of β-cells to α-cells was comparable to that in control mice (Supplementary Fig. 2B and C). Similarly, in MIN6 cells, 24-h ator treatment reduced the cell size (Fig. 2F and G) but did not affect the cell cycle (Supplementary Fig. 2D and B). However, electron microscopy (EM) examination of the islet ultrastructure showed that ator treatment increased the number of immature granules \( (P = 0.02) \) (Fig. 2H) and tended to decrease the granule diameter \( (P = 0.007) \) (Fig. 2I and J) in β-cells, implying that ator treatment might compromise insulin biosynthesis.

Ator Inhibited mTOR Signaling and Key Pancreatic Transcription Factor Expression

To further elucidate how intracellular β-cell signaling was affected by ator, we studied the transcriptome profile of isolated primary islets by RNA sequencing. Overall, 2,632 differentially expressed genes (DEGs; fold change >2.0 and \( q < 0.05 \)) were found, including 1,602 with upregulation and 1,030 with downregulation (Fig. 3A). A principal component analysis indicated significantly different gene expression profile between islets treated with or without ator (Fig. 3B). Gene ontology analysis and pathway analysis suggested that metabolic processes, vesicle-mediated transport, immune responses, secretion regulation, and mTOR signaling were significantly altered by ator treatment (Fig. 3C and D). The expression of key components of mTOR signaling, such as Rheb, Akt3, Rps6ka6, and Deptor, was significantly changed after ator treatment (Fig. 3B). The phosphorylation of S6 at S240/244 (mTORC1) and Akt at S473 (mTORC2) as well as the membrane staining of PKCα (mTORC2) in primary mouse islets were significantly altered by ator treatment (Fig. 3F and G and Supplementary Fig. 3A). Furthermore, in MIN6 cell culture, ator treatment inhibited mTOR signaling in a dose-dependent manner (Fig. 3H and Supplementary Fig. 3B). Other types of statins, such as lovastatin, rosuvastatin, and fluvastatin, exerted similar effects on mTOR signaling in MIN6 cells. The effect of
the hydrophilic statin rosuvastatin was less pronounced than that of the lipophilic statins on β-cells (Fig. 3I and Supplementary Fig. 3C).

The islet RNA-seq data showed that ator treatment downregulated the expression of a number of key β-cell transcription factors (TFs) and secretion-related genes, such as *MafA* and homeobox 6.1 (*Nkx6.1*), and upregulated the expression of disallowed genes, such as hexokinase 1 (*HK1*), c-Maf (*Maf*), and transglutaminase 2 (*Tgm2*) (Fig. 4A) (confirmed by RT-PCR shown in Figure 2—Ator affected pancreatic islet morphology. A: Representative immunohistochemistry images of pancreas sections stained with eosin (red) and insulin (brown) (n = 5). B: Total β-cell mass (n = 5, normalized to body weight [BW]). C: Quantification of β-cell size obtained from Glut2-immunolabeled pancreatic sections (n = 5–6, 500 cells per animal). D: Representative images of immunofluorescence staining for Glut2 (red) and insulin (green) in the HFD and HFD+ator groups. E: β-Cell proliferation rate calculated by Ki67-positive β-cells (n = 5–6). F: Quantification of MIN6 cell size using flow cytometry FSC (n = 4). G: Histogram of MIN6 cell FSC using flow cytometry (n = 4). H: Quantification of the percentage of mature and immature vesicles (n = 3) in β-cells from EM images. Quantification of vesicle diameter (I) and representative EM images (J) of pancreatic islets (n = 3). Blue arrow, mature granule; red arrow, immature granule. Data are presented as the mean ± SEM. *P < 0.05, **P < 0.01; unpaired two-tailed Student t test.
Figure 3—Transcriptome profile of pancreatic islets after ator treatment. A: Volcano plot of RNA-seq data from mouse islets ($n = 3$). B: Principal component analysis (PCA) of DEGs ($n = 3$). C: Gene ontology (GO) analysis of genes in B. D: Pathway analysis of all DEGs. The top 20 significantly changed pathways are presented ($n = 3$). E: Heat map of DEGs in the mTOR signaling pathway ($n = 3$). Color key = z score of normalized relative expression of genes in the row. F: Representative images of pancreatic sections stained for pS6 S240/244 or PKCθ (red), insulin (green), and DAPI (blue). Original magnification $\times 400$ ($n = 5–6$). G: Western blot analyses of pS6 S240/244 and pAkt S473 in mouse islets ($n = 4$). H: Western blot of pS6 S240/244 and pAkt S473 in MIN6 cells treated with ator at different dosages ($n = 4–5$). I: Western blot of pS6 S240/244 and pAkt S473 in MIN6 cells treated with ator, lovastatin (lova), rosuvastatin (rosu), and fluvastatin (flu) ($n = 4–5$).
Supplementary Fig. 3). Furthermore, reduced MafA expression after ator treatment was confirmed by the reduced number of MafA/insulin double-positive cells (Fig. 4B and C) and the diminished islet MafA transcription level identified by RT-PCR (Fig. 4D). Accordingly, ator dose-dependently inhibited MafA expression in MIN6 cells (Fig. 4E and Supplementary Fig. 3E), and treatment with statins, such as lovastatin, ator, fluvastatin, and rosuvastatin, all significantly inhibited MafA levels (Fig. 4F and Supplementary Fig. 3F). The altered expression of β-cell TFs such as MafA and Nkx6.1 might be secondary to mTOR signaling attenuation (21,22).

Supplementation With GGPP Rescued Ator-Attenuated β-Cell Function and mTOR Signaling
To test the potential effect of decreased circulating cholesterol levels on β-cells after ator treatment, we examined the changes in mTOR signaling and TF expression after MβCD treatment, which efficiently diminished intracellular cholesterol in MIN6 cells (Supplementary Fig. 4A–C). No significant alterations in mTOR signaling and MafA levels were found after MβCD treatment compared with the controls (Supplementary Fig. 4D–F); thus, we thought that the decreased circulating cholesterol might not cause the changes of β-cells induced by ator.
Figure 5 — GGPP rescues the inhibitory effect of ator in β-cells. A: Schematic of the mevalonate pathway regulated by ator. B and C: Western blot analysis of raptor, rictor, pS6 S240/244, and pAkt S473 protein levels in MIN6 cells supplemented with FPP (10 μmol/L) or GGPP (10 μmol/L) after 24 h of ator (10 μmol/L) incubation (n = 3–9). D: RT-PCR analysis of Glut2, Gck, MafA, and MafB after 24 h of ator incubation in MIN6 cells supplemented with FPP or GGPP (n = 6–7). E: Western blot analysis of MafA in MIN6 cells supplemented with FPP or GGPP after 24 h of ator incubation (n = 5–9). F: GSIS of 3 × 10⁶ MIN6 cells pretreated with ator or supplemented with FPP or GGPP. Culture conditions with 3.3 mmol/L, 16.7 mmol/L, and 33.3 mmol/L glucose (n = 4). The protein levels of MafA, pAkt S473, and pS6 S240/244 (n = 5) (G), mRNA levels of MafA (n = 5) (H), and GSIS (n = 4–8) (I) in MIN6 cells after lovastatin (10 μmol/L) incubation with FPP or GGPP supplementation. The protein levels of MafA, pAkt S473, and pS6 S240/244 (n = 4) (J), mRNA levels of MafA (n = 4–6) (K), and GSIS (n = 4–8) (L) in MIN6 cells after fluvastatin (10 μmol/L) incubation with FPP or GGPP supplementation. The protein levels of MafA, pAkt S473, and pS6 S240/244 (n = 4) (M), mRNA levels of MafA (n = 5) (N), and GSIS (n = 4–8) (O) in MIN6 cells after rosuvastatin (10 μmol/L) incubation with FPP or GGPP supplementation. Data are presented as the mean ± SEM. *P < 0.05, **P < 0.01, ***P < 0.001; #P < 0.05, ##P < 0.01, ###P < 0.001 vs. ator; unpaired two-tailed Student t test, one-way ANOVA for four groups. G, glucose; GSIS, glucose-stimulated insulin secretion; SQS, squalene synthase.
Recent studies have found that statins directly affect adipose browning or inflammation by inhibiting the synthesis of isoprenoids, which are the intermediates of the mevalonate pathway (11,12) (Fig. 5A). We then tested this pathway in β-cells. Surprisingly, supplementation with FPP or GGPP induced partial or almost complete recovery of mTOR signaling (Fig. 5B and C and Supplementary Fig. 5A and B) and MafA expression (Fig. 5D and E and Supplementary Fig. 5B), respectively, after ator treatment. Moreover, GGPP and FPP supplementation recovered the impaired GIIS in MIN6 cells after 24 h of ator treatment (Fig. 5F). Similar results were observed after treatment with other statins, such as lovastatin (Fig. 5G–I and Supplementary Fig. 5C), fluvastatin (Fig. 5J–L and Supplementary Fig. 5D), and rosuvastatin (Fig. 5M–O and Supplementary Fig. 5E). The hydrophilic statin was less potent than the lipophilic statins. Thus, our data suggested that the disrupted β-cell function, as well as mTOR signaling and TF expression, induced by ator could be rescued by isoprenoid restoration.

### sGPs Link the Regulation of Statin/GGPP to mTOR Signaling

We then sought to determine why disrupted isoprenoid synthesis by ator mediated its negative effect on β-cells. Some prenylation-modulated sGPs are important in the regulation of mTOR signaling (20). The prenylation of sGPs by GGPP requires geranylgeranyltransferases (GGTases) GGTase-I and GGTase-II (also called Rabggtb) (36). The use of a pharmacological inhibitor, GGTI-298 (to specifically inhibit GGTase-I) (Supplementary Fig. 6A–D), or siRNA to silence GGTase-II expression (knockdown of the β subunit of GGTase-II, siRabggtb) (Supplementary Fig. 6E–G) showed similar inhibitory effects on mTOR signaling, key pancreatic TF expression, and β-cell function as ator treatment. Thus, impaired sGP prenylation after statin treatment might disrupt sGP function, which could inhibit mTOR signaling and subsequently cause β-cell deficiency.

Interestingly, islet mRNA sequencing showed that the expression levels of multiple members of all five sGP family groups (Ras, Rho, Rab, Arf, and Ran) were significantly altered by ator treatment. Among these sGPs, Rab5a was the most affected (Fig. 6A). Rab5a is an endosome-anchored small GTP-binding protein that has been reported to regulate mTOR signaling (37). Ator treatment reduced both the RNA and protein levels of Rab5a in mouse islets (Fig. 6B and C and Supplementary Fig. 7A) and MIN6 cells (Fig. 6E and F and Supplementary Fig. 7B). In addition, membrane-associated Rab5a, the active form (27), seemed to be more affected by ator than cytosolic Rab5a (Supplementary Fig. 7C and D). GGPP supplementation was sufficient to rescue the ator-induced reduction in Rab5a levels (Fig. 6E and F and Supplementary Fig. 7B). Furthermore, the expression of Glut2, Gck, MafA, MafB (Fig. 6G), and key mTOR downstream proteins (Fig. 6H and Supplementary Fig. 7E) was also reduced by knocking down Rab5a in MIN6 cells. Notably, the expression of another sGP known to target mTORC1, Rheb (38), was not affected by ator treatment (Supplementary Fig. 7F–H).

### DISCUSSION

In the current study, we delineated the mechanism of the direct effects of chronic statin treatment on β-cells. In HFD mice treated with ator for 4 months, we found reduced β-cell mass and size, decreased quantities of mature insulin granules in vivo, and impaired GIIS. The inhibition of HMG-CoA reductase by ator impaired the production of isoprenoid, which disrupted the expression of sGPs, such as Rab5a, thus inhibiting mTOR signaling and the activity of key pancreatic TFs such as MafA. GGPP supplementation restored the inhibited mTOR signaling, MafA activity, and β-cell function induced by ator. Thus, we hypothesize that ator negatively regulates β-cell function by inhibiting GGPP production.

Suppression of mTOR activity impairs β-cell mass and functional maturity (21–24,39). Ator treatment attenuated the mTOR signaling and induced similar phenotypes in HFD-fed mice and MIN6 cells with abolished mTOR signaling (22,40,41). mTOR regulates key pancreatic TFs (21,22), including MafA (42), that maintain β-cell identity and functionality and were substantially suppressed by ator treatment. Thus, mTOR signaling might mediate the negative effect of statins on β-cell TFs and function. However, why and how can mTOR signaling be affected by ator? A few studies have shown that statins affect mTOR signaling via intermediates in the mevalonate pathway other than cholesterol (12,43,44). In this study, we first showed that GGPP supplementation significantly rescued the disrupted mTOR signaling in MIN6 cells treated with ator. Thus, based on current evidence, we thought that the inhibitory effect of ator on mTOR signaling in β-cells could pertain to attenuated isoprenoid synthesis.

Our study further provides evidence that GGPP production could be critical for mediating statin-induced pancreatic β-cell dysfunction, highlighting the important role of the mevalonate pathway in regulating β-cell function. Jiang et al. (19) reported that Ggpps-specific deletion in pancreatic β-cells inhibits the geranylgeranylation and membrane association of Rab27a, causing impaired insulin secretion. Thus, there could be a wide range of sGPs exerting redundant effects to maintain mTOR activity and β-cell functional mass that might respond simultaneously to statin treatment. Therefore, it was unexpected, but conceivable, that the RNA sequencing data revealed that multiple sGPs in islets, belonging to all five subfamilies, were transcriptionally inhibited by ator treatment. Among these sGPs, Rab5a was most significantly reduced after ator treatment, particularly the membrane-associated portion (active Rab5a). Known for regulating endolysosomal system biogenesis (45), Rab5a is located upstream of mTORC1 signaling and is involved in
mTORC1-targeted autophagic processes in vascular smooth muscle cells (37). Our study could be the first to report that this protein underlies the detrimental effect of statins on pancreatic β-cells. Hence, statins could regulate mTOR signaling by inhibiting sGPs via disrupting prenylation, and subsequently affect the expression of the main pancreatic TFs.

Notably, the reductions in sGPs and mTOR signaling may not be the only intracellular effects of ator. Oxygenated derivatives of cholesterol (e.g., 24S-OHC, 25-OHC,
27-OHC, 7-ketocholesterol, and 7β-OHC) can also be decreased by statin treatment (46,47). Oxygenated cholesterol affects several nuclear receptors, such as LXRs and SREBPs, which regulate a wide range of metabolic processes, including islet function (48–52). However, the role of these genes in islets is still controversial and needs to be further clarified. Therefore, future studies are required to reliably quantify oxygenated cholesterol and examine the potential role of these metabolites in the effects of statins.

Importantly, we found similar changes in β-cells induced by three other statins, indicating that sGP/mTOR regulation might be the common intracellular signaling pathway targeted by statins. Notably, we found that the degree of effect of statins on this pathway and β-cell function varied with their hydrophilicity. The hydrophilic statin rosvuastatin showed the least negative effect on MIN6 cells among all tested statins at equivalent dosages. This result is consistent with the study of Balaz et al. (11), showing that the lipophilicity of different statins influences their effects on inhibiting UCP1 in human multipotent adipose tissue–derived stem cells. Thus, our results may provide important clinical implications for the choice of the statins in patients with a high risk for type 2 diabetes.

In conclusion, we demonstrated that ator treatment reduced HMG-CoA reductase–related isoprenoid production in pancreatic islets and hence impaired β-cell mTOR signaling and functional mass, thus inducing diabetes (Supplementary Fig. 8). Supplementation with isoprenoids, such as GGPP, might help to minimize and prevent the risk of new-onset diabetes induced by statins. Although the degree of statin-induced hyperglycemia was moderate, our study provides a better understanding of the role of the mevalonate pathway in regulating β-cell function and will facilitate the discovery of new druggable targets in this pathway for type 2 diabetes. Future studies on genetically engineered mice are required to confirm our findings.

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