



N-glycosylation of somatostatin receptor type 2 protects rats from acute pancreatitis

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Background: The growth hormone inhibitor somatostatin and its analogs are promising therapeutic agents for acute pancreatitis. However, the therapeutic effects remain controversial. Somatostatin analogs preferentially bind to somatostatin receptor 2 (SSTR2), and this study aimed to investigate whether N-glycosylation affects SSTR2 stability, membrane trafficking, and signal transduction.

Methods: Western blot analysis following PNGase F digestion was performed to confirm N-glycosylation of SSTR2 in rat pancreatic acinar AR42J cells. Rats were subjected to 4 hourly intraperitoneal injections of cerulein (50 µg/kg) plus LPS (5 µg/mL) to induce acute pancreatitis. Mass spectrometry was conducted to identify the glycosylation sites of SSTR2, and immunofluorescent staining was carried out to examine the localization of wild-type and asparagine 9 (N9)Q-mutant SSTR2. Proteasome inhibitor MG132 was employed to assess the stability of SSTR2, and overexpression of wild-type or N9Q-mutant SSTR2 was used to examine the role of N-glycosylation in SSTR2 signal transduction *in vitro* and its therapeutic effect on pancreatitis in rats.

Results: SSTR2 protein in AR42J cells was N-glycosylated at the N9 residue. Wild-type SSTR2 localized in the plasma membrane whereas N9Q-mutant SSTR2 was retained in the endoplasmic reticulum (ER). MG132 rapidly restored the ectopic expression of mutant but not wild-type SSTR2 protein in AR42J cells. Overexpression of wild-type but not N9Q-mutant SSTR2 activated NF-κB signaling and facilitated nuclear transportation of p65 in AR42J cells upon cerulein plus LPS stimulation. Furthermore, pretreatment with wild-type but not N9Q-mutant SSTR2-overexpressing vectors significantly ameliorated biochemical parameters and pancreatic histological impairments in rats with pancreatitis.

Conclusions: N-glycosylation of SSTR2 is essential for membrane localization, stability, and signal transduction of SSTR2 in pancreatic cells, playing a protective role in experimental acute pancreatitis.

Keywords: Acute pancreatitis; somatostatin receptor type 2; NF-κB; glycosylation; inflammation

Submitted Sep 09, 2021. Accepted for publication Nov 12, 2021.

doi: 10.21037/atm-21-5249

View this article at: <https://dx.doi.org/10.21037/atm-21-5249>

Introduction

Acute pancreatitis is an inflammatory disorder, and is the most common gastrointestinal disease leading to hospital admission (1). The mortality rate of acute pancreatitis ranges from 2.22% to 45.63%, depending

on the severity of the disease (2), while activation of the pancreatic enzymes in acinar cells leading to autodigestion of pancreatic tissue triggers the disease (3). The growth hormone inhibitor somatostatin (SST) is an endogenous peptide and known to regulate diverse cellular processes

such as neurotransmission, hormone secretion and cell proliferation. Somatostatin and its analogs are promising therapeutic agents for acute pancreatitis through inhibiting pancreatic enzyme secretion (4). At the same time, SST can also reduce visceral blood flow and promote the recovery of gastrointestinal function. A variety of mechanisms work together to reduce the activities of trypsin and pepsin in SAP patients, and then reduce the damage and inflammatory reaction of pancreas and gastric tissue in SAP patients. However, a clinical trial including 302 patients with moderate to severe acute pancreatitis showed somatostatin had no effects on the mortality rate, rate of newly developed complications, duration of the pain, rate of surgical interventions, and length of the hospital stay (5). Thus, it is necessary to explore the factors that affect the therapeutic response to somatostatin in acute pancreatitis.

Somatostatin and its analogs exert their functions by binding to G-protein-coupled somatostatin receptors (SSTRs) that comprise five subtypes (SSTR1–5), among which SSTR2 is predominantly responsible for endogenous somatostatin-mediated regulation of hormone secretion (6). Clinically available somatostatin analogs preferentially binds to SSTR2 (7–9), and its expression positively correlates with the outcome of somatostatin or somatostatin analog treatment in acromegaly (7) and growth hormone-secreting pituitary adenomas (10). Moreover, due to the abundant SSTR2 expression in pancreatic inflammatory and tumor cells, somatostatin-related compounds achieve promising therapeutic effects against pancreatic disease at least partially through SSTR2-induced activation of the inhibitor κ B kinase (IKK)/NF- κ B signaling cascade (11–13). Considering SSTR2 belongs to the superfamily of G-protein-coupled receptors, we speculated that its stability and membrane trafficking might affect the response to somatostatin therapy in patients with acute pancreatitis. However, the regulatory mechanism of the stability and membrane trafficking of SSTR2 remains unclear. Most membrane proteins, including SSTRs, undergo N-glycosylation (14), and SSTR2 displays typical seven-transmembrane segments and contains four putative N-glycosylation sites (N9, N22, N29, and N32). In Western blot experiments, the SSTR2 protein band migrates between 70 and 80 kDa, suggesting SSTR2 protein may be unglycosylated or N-glycosylated at one or more asparagine (Asn) residues (15). N-glycans are involved in the folding, stability, trafficking, and localization of the proteins, and consequently modulate their functions (16). N-glycosylation also affects ligand binding, and mutation or truncation of its sites in SSTR3

results in significantly reduced binding to somatostatin and impaired signal transduction (17). Thus, we hypothesized that N-glycosylation of SSTR2 might affect its stability, membrane localization, and downstream signal transduction in pancreatic inflammation.

To test our hypothesis, we confirmed that endogenous SSTR2 is only glycosylated at N9 residue in AR42J cells, and the absence of N9 glycosylation blocks SSTR2 trafficking and membrane localization, then we examined the role of N-glycosylation of SSTR2 in cerulein plus lipopolysaccharide (LPS)-stimulated pancreatic acinar cells as well as in a pancreatitis mouse model. Our data suggest that the presence of N-glycan is required for the stability, membrane trafficking, and downstream signal transduction of SSTR2, protecting rats from cerulein plus LPS-induced pancreatic inflammation. We present the following article in accordance with the ARRIVE reporting checklist (available at <https://dx.doi.org/10.21037/atm-21-5249>).

Methods

Animals

The animal study was approved by the Chinese National Committee for the Use of Experimental Animals for Medical Purposes, Jiangsu Branch [JN.No20190930c0480120(233)], and all procedures were carried out following the Guide for the Care and Use of Laboratory Animals (National Research Council, 1996, USA). A total of 20 male Sprague-Dawley rats, 6–8 week-age, weighing 180–200 g were purchased from the Beijing Vital River Experimental Animals Technology Co., Ltd. [Beijing, China, SCXK (Jing) 2016-0006], and maintained under specific pathogen-free conditions.

Cell culture and treatment

Rat pancreatic acinar AR42J cells were obtained from the China Center for Type Culture Collection (Wuhan, China) and maintained at 37 °C in minimum essential medium (MEM) (Hyclone, Logan, UT, USA), which was supplemented with 10% fetal bovine serum (Hyclone), 100 U/mL penicillin and 100 μ g/mL streptomycin, and the air contains 5% carbon dioxide. For cerulein plus LPS stimulation, AR42J cells were plated into 60-mm dishes and incubated for 24 h then stimulated with 10 nmol/L cerulein (Sigma-Aldrich, St. Louis, MO, USA) and 10 mg/L LPS (Sigma-Aldrich) for 0, 4, 8, 12, or 24 h (18) with phosphate-

buffered saline (PBS) as a vehicle. Tunicamycin is a potent protein N-glycosylation inhibitor that has frequently been used to manipulate protein glycosylation in cells. To inhibit N-glycosylation, AR42J cells were cultured in medium containing 1 µg/mL tunicamycin (sigma-Aldrich) for 48 h, and for proteasome inhibition, cells were treated with 10 µM MG132 (Sigma-Aldrich) for 0, 2, or 4 h (19).

Vectors, site-directed mutagenesis, and transfection

A pcDNA3 plasmid overexpressing rat SSTR2 was purchased from the PPL company. To generate an asparagine-to-glutamine (N9Q) mutation in SSTR2 protein, a mutant SSTR2 plasmid was constructed by PCR-based mutagenesis using long-Taq-DNA polymerase (BIOSCI) and primers 5'-TGCTCTAGAATGGAGTTGACCTCTGAGCAGTTCAATCAAGGGAGC-3' (sense) and 5'-CCGGAATTCCAGATACTGGTTTGGAGGTT-3' (antisense). The plasmids were transiently transfected into AR42J cells using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions.

Acute pancreatitis mouse model

Acute pancreatitis was induced in rats as previously described (3,20), and the experimental condition was as follows: the temperature was 22±2 °C; the humidity was 50%±5%; all mouse were fed with free drinking water and standard food; we maintained mouse circadian rhythm by alternating lighting. Briefly, rats were randomized to four groups: a pretreated with empty vectors (n=5) + PBS group, pretreated with empty vectors (n=5) + LPS group; vectors expressing wild-type SSTR2 (n=5) + LPS group, and vectors expressing N9Q-mutant SSTR2 (n=5) + LPS via caudal vein injection group. At 72 h after pretreatment, except for rats in the pretreated with empty vectors group, all others were subjected to 4 hourly intraperitoneal injections of cerulein (50 µg/kg) plus LPS (5 µg/mL) to induce acute pancreatitis. The control rats were administered the same volume of PBS at the same time points. Rats were sacrificed at several time intervals from 4 to 36 hours after the last intraperitoneal injection with cerulein and LPS.

Western blot analysis

Total proteins were obtained from AR42J cells using RIPA buffer, and to remove the N-glycan, 50 µg protein

samples were incubated with 50 units of PNGase F (New England Biolabs, USA) at 37 °C for 4 h according to the manufacturer's instruction. Peptide N-glycosidase F (PNGase F) is the most widely used enzyme for releasing N-glycans from glycoproteins in glycoanalytical workflows due to the broadest specificity and ability to liberate the most diverse range of N-glycan structures from glycoproteins. The protein samples were separated by SDS-polyacrylamide gel electrophoresis and transferred to a nitrocellulose membrane, and after blocking with 5% non-fat milk in Tris-buffered saline-Tween 20 (TBST) for 2 h at room temperature, the membrane was washed with TBST then incubated with primary antibody against SSTR2 (1:500), IκBα (1:500), p65 (1:500), p-p65 (1:1,000), or β-actin (1:1,000) at 4 °C overnight. All the above antibodies were purchased from Santa Cruz Biotechnology (Dallas, TX, USA). The membrane was then incubated with horseradish peroxidase conjugated secondary antibody (1:10,000; Santa Cruz Biotechnology) for 2 h at room temperature. The protein bands were detected using an enhanced chemiluminescence system (Thermo Fisher Scientific, Waltham, MA, USA), and their densities were quantified using Image J (NIH, MD, USA).

Immunofluorescence assay

At 8 h after cerulein plus LPS treatment, we fixed AR42J cells with 4% paraformaldehyde in PBS for 1 h at room temperature. then they were blocked with 10% donkey serum for 2 h at room temperature. Then we incubated the cells with primary antibody against myc (1:500; Santa Cruz Biotechnology), calnexin (1:500; Santa Cruz Biotechnology), or p65 (1:500; Santa Cruz Biotechnology) in 1% bovine serum albumin overnight at 4 °C, followed by incubation with Alexa Fluor 568- or Alexa Fluor 488-conjugated antibody (Invitrogen) for 2 h at room temperature. The nucleus was stained using 5 µg/mL Hoechst, and images were captured using a laser scanning confocal microscope (LSM710; Zeiss, Germany).

Liquid chromatography-electrospray ionization-mass spectrometry (LC-ESI-MS) analysis

To identify the N-glycosylation site of SSTR2, myc-his-SSTR2 proteins were purified using a Ni column and eluted with imidazole solution. The purified SSTR2 proteins then underwent PNGase F-mediated removal of N-glycan, dialysis on a C18 column, and trypsin digestion. The

digested sample was analyzed using an LC-ESI-MS system consisting of an Aquasil C-18 precolumn (30 mm × 0.32 mm, 5 mm, Thermo Scientific), a BioBasic C18 analytical column (150 mm × 0.18 mm, 5 mm, Thermo Scientific), a Waters CapLC, a Rheodyne 10-port valve, and a Waters Q-TOF Ultima with standard ESI-source, as previously described (21). The mobile phase A was composed of 65 mM ammonium formate (pH 3.0), and phase B was composed of 80% acetonitrile in solvent A. The capillary voltage and cone voltage were 3.2 kV and 35 V, respectively, and the source temperature and desolvation temperature were 100 and 120 °C, respectively. Peptides were trapped in the precolumn before being separated on the analytical column at a flow rate of 5 µL/min.

Measurements of biochemical parameters

Rats were sacrificed at 4 to 36 h after cerulein plus LPS administration. Blood samples were immediately collected from the vena cava, and serum samples were obtained by centrifuging the blood samples at 3,500 rpm for 10 min. The serum amylase and lipase activities were measured using an amylase activity assay kit and lipase activity assay kit (Sigma-Aldrich), respectively, according to the manufacturer's protocols. The serum concentrations of TNF- α , IL-6, and myeloperoxidase (MPO) were measured using corresponding ELISA kits (Excell, Shanghai, China) following the manufacturer's instructions. All samples were analyzed three times using a microplate reader (BioRad, Hercules, CA, USA).

Haematoxylin & eosin (H&E) staining

Fresh rat pancreas specimens were fixed in 4% paraformaldehyde in PBS, embedded in paraffin, and cut into 5-mm-thick sections. H&E staining was performed following standard procedures, and the histological grading of edema, leukocyte infiltration, and vacuolization was conducted using a scale ranging from 0 to 3 as previously described (22).

Statistical analysis

Statistical analysis was performed using Stata 7.0 (StataCorp, College Station, TX, USA). Data are processed with mean \pm standard error. The differences among groups were determined by one-way analysis of variance, followed by Tukey's post hoc multiple comparison test, and a

P value <0.05 was considered statistically significant. All experiments were performed in triplicate.

Results

SSTR2 is glycosylated at N9 residue in AR42J cells

To examine the glycosylation state of SSTR2 in AR42J cells, we treated them with the N-glycosylation inhibitor tunicamycin. Western blot analysis revealed that the SSTR2 protein band shifted downward due to the decreased molecular weight in response to tunicamycin treatment (*Figure 1A*), and digestion of the cell lysates with endoglycosidase PNGase F caused a similar band shift (*Figure 1B*). These results suggest that endogenous SSTR2 in AR42J cells is N-glycosylated.

We then immunoprecipitated SSTR2 protein to identify the glycosylation sites, and the MS analysis showed that the N9 residue was a potential N-glycosylation site in SSTR2 (*Figure 1C*). Compared with the wild-type SSTR2 protein band, the N9Q-mutant SSTR2 protein band migrated downward but did not migrate further after PNGase F digestion (*Figure 1D*). These results suggest that endogenous SSTR2 is only glycosylated at N9 residue in AR42J cells.

N9 glycosylation is required for SSTR2 protein trafficking and stability

Since N-glycosylation contributes to protein trafficking, localization, and stability (23), we examined the subcellular localization and stability of overexpressed wild-type and N9Q-mutant SSTR2 proteins in AR42J cells. Immunofluorescent staining showed a widespread distribution of the wild-type SSTR2 protein in the plasma membrane and colocalization of N9Q-mutant SSTR2 protein with the endoplasmic reticulum (ER) marker calnexin (*Figure 2A*). This finding suggests that the absence of N9 glycosylation blocks SSTR2 trafficking and membrane localization. Furthermore, Western blot analysis revealed that AR42J cells transfected with wild-type SSTR2 plasmid expressed significantly higher levels of fusion proteins than those transfected with N9Q-mutant SSTR2 plasmid. Treatment with proteasome inhibitor MG132 rapidly restored the expression of N9Q-mutant SSTR2 protein in AR42J cells (*Figure 2B*), suggesting these proteins are more sensitive to proteasome-mediated degradation than wild-type SSTR2. Taken together, these results suggest that N9-

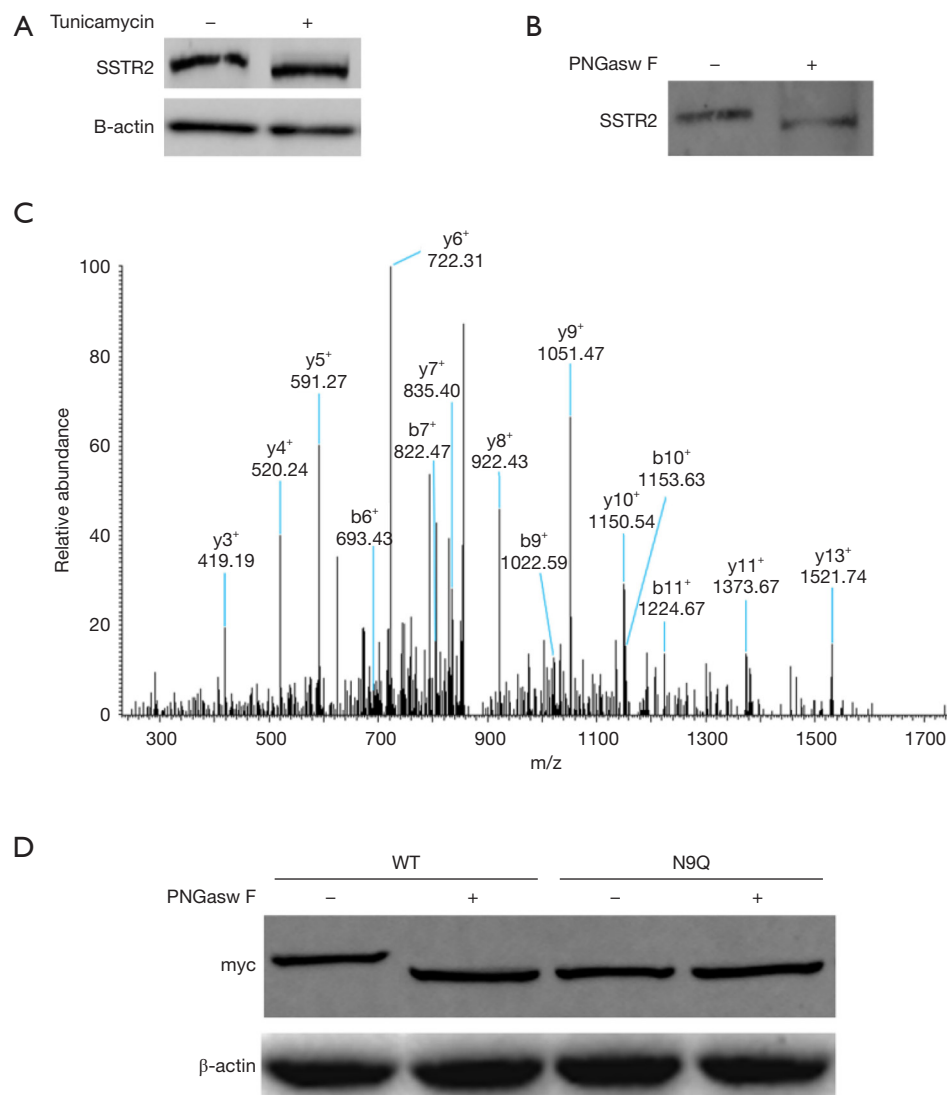


Figure 1 N-glycosylation of SSTR2 in AR42J cells. (A) AR42J cells were treated with 1 $\mu\text{g}/\text{mL}$ tunicamycin for 24 h. (B) Cell lysates of AR42J cells were denatured and incubated with 50 units of PNGase F for 4 h. Western blot analysis was performed to determine SSTR2 protein expression. β -actin was used as an internal control. (C) LC-ESI-MS was performed to identify N-glycosylation sites in SSTR2 protein. (D) AR42J cells were transiently transfected with vectors overexpressing wild-type or N9Q-mutant SSTR2. Cell lysates were denatured, incubated with PNGase F for 4 h, and subjected to Western blot analysis. SSTR2, somatostatin receptor 2; WT, wildtype; N9Q, asparagine-to-glutamine mutation.

glycosylation is required for SSTR2 protein stability and membrane localization in pancreatic cells.

N9 glycosylation is required for SSTR2 to activate NF- κ B signaling in inflammatory AR42J cells

In order to assess the contribution of N-glycosylation in SSTR2-mediated activation of NF- κ B signaling, we

provided AR42J cells with cerulein plus LPS to induce an inflammatory response. Western blot analysis demonstrated that cerulein plus LPS stimulation activated NF- κ B signaling in AR42J cells, as evidenced by a significant decline of NF- κ B suppressor $\text{I}\kappa\text{B}\alpha$ (18) protein expression starting 8 h after treatment (*Figure 3A*). Compared with the control, overexpression of wild-type SSTR2 significantly attenuated protein expression of $\text{I}\kappa\text{B}\alpha$ while enhancing protein

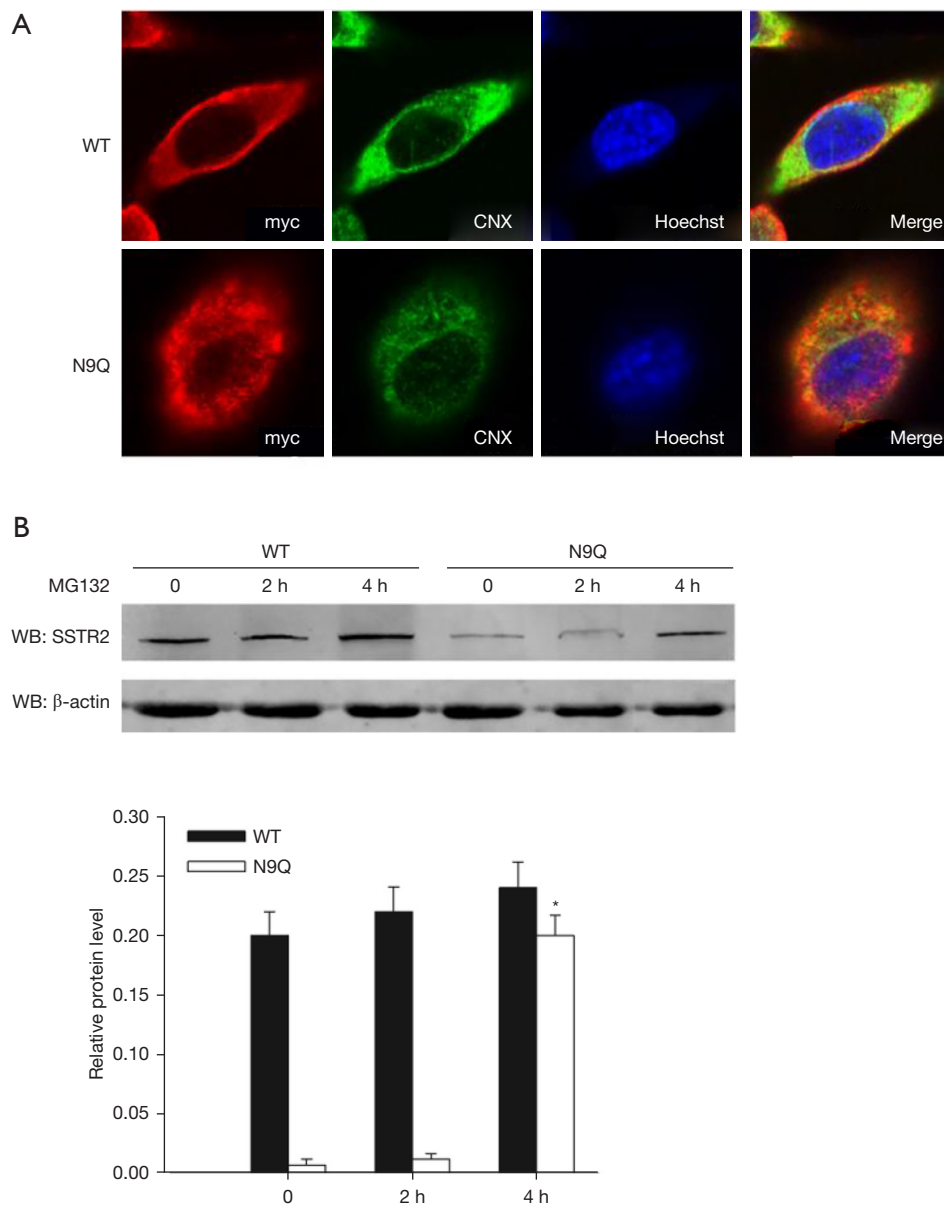


Figure 2 N9 glycosylation was required for SSTR2 protein trafficking and stability. (A) Immunofluorescence staining shows the localization of wild-type and mutant SSTR2 in AR42J cells. Cells were stained with anti-myc (red) and anti-CN_X (green) antibodies. (B) At 36 h after transfection with corresponding vectors as indicated, AR42J cells were incubated with MG132 for an additional 0, 2, or 4 h. Western blot analysis was performed to determine the protein expression of SSTR2. β -actin was used as an internal control. Data are expressed as the mean \pm SEM. * P <0.05, *vs.* the 0 or 2 h N9Q group; n =3. WT, wildtype; N9Q, asparagine-to-glutamine mutation; SSTR2, somatostatin receptor 2; CN_X, calnexin; SEM, standard error of the mean.

expression of p-p65 in AR42J cells at 8 h after exposure to cerulein plus LPS, suggesting that overexpression of wild-type SSTR2 activates NF- κ B signaling in AR42J cells upon cerulein plus LPS stimulation. On the other hand, overexpression of N9Q-mutant SSTR2 showed no effects

on I κ B α and p-p65 expression compared with the control (Figure 3B) and failed to facilitate nuclear transportation of p65 compared with wild-type SSTR2 (Figure 3C). These findings suggest N9 glycosylation is required for SSTR2 to activate NF- κ B signaling in inflammatory AR42J cells.

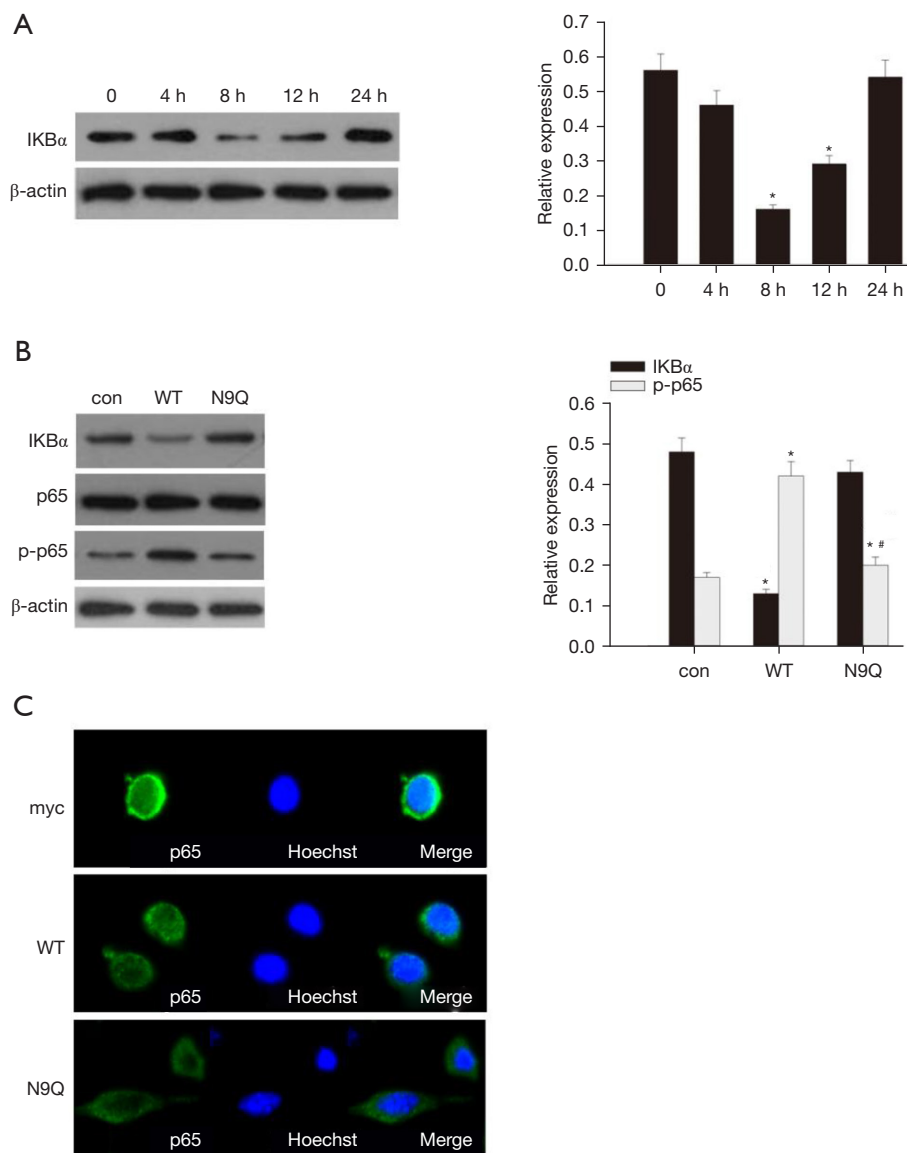


Figure 3 N9Q-mutant SSTR2 failed to activate NF- κ B signaling in AR42J cells. (A) AR42J cells were stimulated with cerulein (10 nmol/L) plus lipopolysaccharide (LPS; 10 mg/L) for 0, 4, 8, 12, or 24 h. Western blot analysis was performed to determine the protein levels of I κ B α . Data are presented as the mean \pm SEM. * $P < 0.05$, vs. 0 h; $n = 3$. (B) AR42J cells were transfected as indicated for 36 h, followed by stimulation with cerulein plus LPS for additional 8 h. Western blot analysis was performed to determine the protein levels of I κ B α , p65, and p-p65. Data are presented as the mean \pm SEM. * $P < 0.05$, vs. con; # $P < 0.05$, vs. WT; $n = 3$. (C) Immunofluorescent staining was conducted to detect nuclear localization of p65. Representative images are shown, with magnification $\times 400$. WT, wildtype; N9Q, asparagine-to-glutamine mutation; SSTR2, somatostatin receptor 2; con, empty vector; N9Q, asparagine-to-glutamine mutation.

N-glycosylation of SSTR2 protects rats from acute pancreatitis

To explore the role of N-glycosylation of SSTR2 in acute pancreatitis *in vivo*, we pretreated the rats into 4 groups:

control rats ($n = 5$); empty vector ($n = 5$); wild-type SSTR2-overexpressing vectors ($n = 5$); and N9Q-mutant SSTR2-overexpressing vectors ($n = 5$) before cerulein plus LPS administration. As shown in *Figure 4A-4D*, compared with control rats, those treated with empty vector and

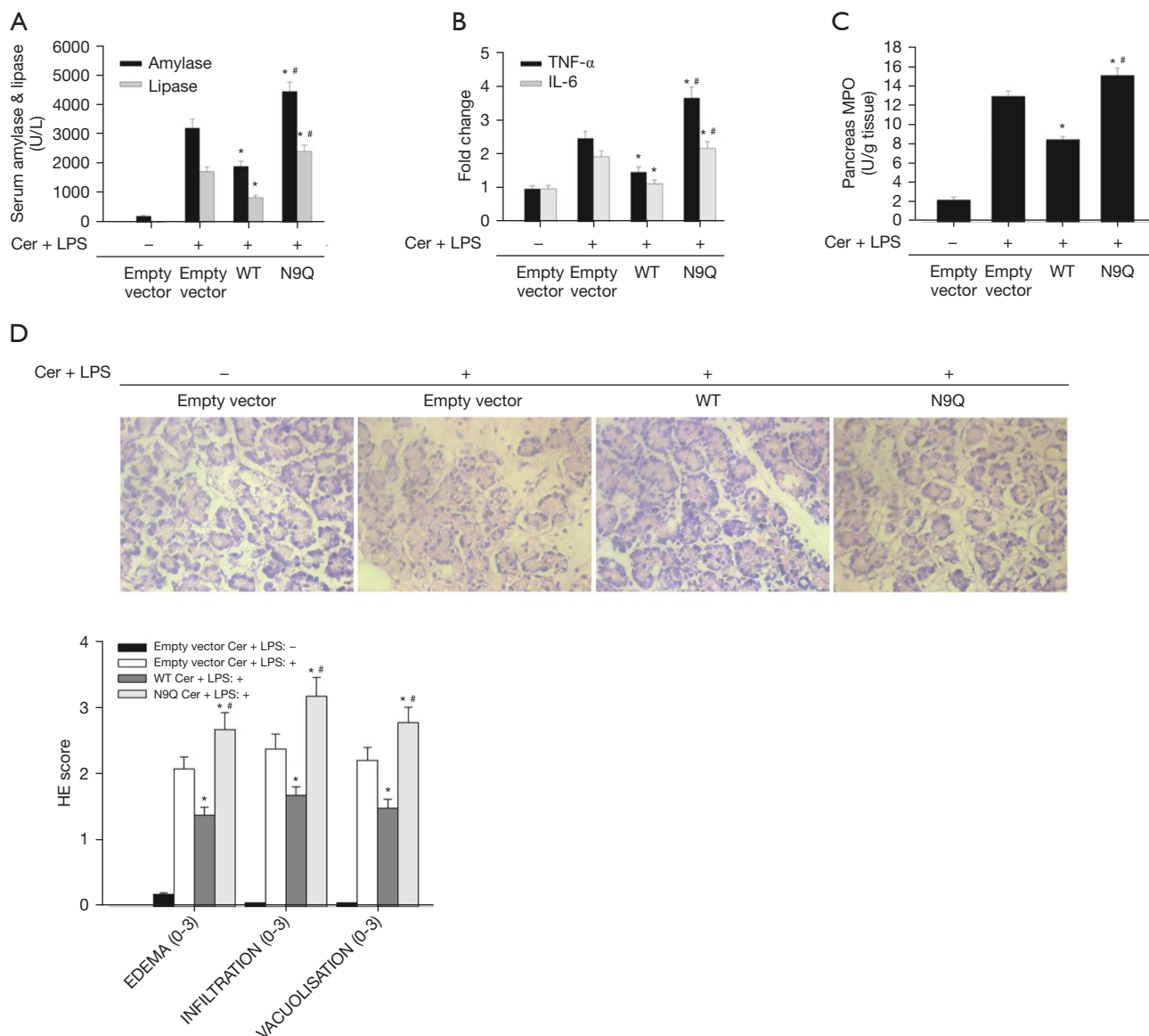


Figure 4 N9Q-mutant SSTR2 aggravated pancreatic inflammation in rats. At 72 h after pretreatment with empty lentiviral vectors, vectors expressing wild-type SSTR2, or vectors expressing N9Q mutant SSTR2, rats were subjected to 4 hourly intraperitoneal injections of cerulein (50 $\mu\text{g}/\text{kg}$) plus LPS (5 $\mu\text{g}/\text{mL}$) to induce acute pancreatitis. The control rats were administered the same volume of sterile PBS at the same time points. The serum levels of amylase & lipase (A), TNF- α , IL-6 (B), and MPO (C) were measured at 8 h after the final injection of cerulein plus LPS. Data are presented as the mean \pm SEM. * $P < 0.05$, vs. empty vector + PBS-treated rats; # $P < 0.05$, vs. WT; $n = 3$. (D) Representative images and scoring of hematoxylin & eosin staining of the pancreatic tissue samples of rats with magnification $\times 200$. Data are presented as means \pm SEM. * $P < 0.05$, vs. empty vector + PBS-treated rats; # $P < 0.05$, vs. WT; $n = 3$. WT, wildtype; N9Q, asparagine-to-glutamine mutation; SSTR2, somatostatin receptor 2; PBS, phosphate-buffered saline; MPO, myeloperoxidase.

cerulein plus LPS exhibited significantly increased serum levels of amylase, lipase, TNF- α , IL-6, and MPO, typical histopathological changes of acute pancreatitis, as well as considerably elevated H&E scores, suggesting

cerulein plus LPS induces acute pancreatitis in the rats. Compared with empty vectors, pretreatment with wild-type SSTR2 partially, but significantly, reversed cerulein plus LPS-induced elevations in serum levels of biochemical

parameters and histopathological changes in the pancreatic tissue. Pretreatment with mutant SSTR2-overexpressing vectors showed contrasting effects. These results suggest that N-glycosylation of SSTR2 ameliorates acute pancreatitis *in vivo*.

Discussion

Somatostatin and its analogs targeting SSTR2 are promising therapeutic agents for pancreatic inflammation and tumor treatment, at least partially via the NF- κ B signaling pathway (13). N-glycosylation plays vital roles in protein maturation and secretion, subcellular trafficking, and signaling transduction of G protein-coupled receptors (24-26). Loss of N-glycans impairs the stability, membrane localization, ligand binding, and signal transduction of SSTRs and consequently affects the response to somatostatin therapy (17). In the present study, we found that endogenous SSTR2 protein was glycosylated at the N9 residue in rat pancreatic acinar AR42J cells. N9Q mutation in SSTR2 impaired the stability of SSTR2, retained the mutant protein in the ER, and blocked cerulein plus LPS-induced activation of NF- κ B signaling. Importantly, overexpression of wild-type but not N9Q-mutant SSTR2 ameliorated cerulein plus LPS-induced pancreatic inflammation. Together, these findings suggest N9 glycosylation is required for the stability, membrane localization, and signal transduction of SSTR2 protein, protecting the pancreas from inflammatory stimuli.

Our results showed that AR42J cells transfected with wild-type SSTR2 plasmid expressed significantly higher levels of fusion proteins than cells transfected with mutant SSTR2 plasmid, suggesting that N9Q mutant fusion SSTR2 protein is considerably less stable than the wild-type protein in cells. The rapid restoration of mutant SSTR2 protein expression after MG132 treatment suggests that proteasomes are responsible for its degradation. Although protein misfolding resulting from amino acid mutation may cause rapid proteasomal degradation (27), our results suggest that loss of N-glycan mainly contributes to the degradation of mutant SSTR2 by proteasomes for the following reasons: (I) by treating AR42J cells with the N-glycosylation inhibitor tunicamycin, SSTR2 was indeed glycosylated, and trafficking of native SSTR2 isoforms beyond the ER was disrupted by inhibition of N-glycosylation. (II) Owing to the similarity of asparagine and glutamine in size and structure, glutamine has been frequently used for asparagine substitution mutation in the study of N-glycosylation in glycoproteins (28,29). The shift of the immunoblot bands

of N9Q-mutant SSTR2 is likely due to the absence of large sugar moiety rather than the replacement of a single amino acid. (III) Glycoproteins with truncated N-glycans may fail to fold properly and retain in the cytoplasm where they are destroyed by proteasomal degradation (16).

NF- κ B signaling plays an important role in immune responses, inflammation, and cellular stress, and SSTR2 has been shown to activate NF- κ B signaling in pancreatic acinar AR42J cells and NIH3T3 cells (13,30). Here, we found that N9Q-mutant SSTR2 failed to activate NF- κ B signaling in AR42J cells, suggesting that disruption of N-glycosylation of SSTR2 blocks the downstream signal transduction in pancreatic cells.

In conclusion, we demonstrated that N-glycosylation was essential for the stability, membrane localization, and signal transduction of SSTR2 in pancreatic cells. Loss of N-glycan disrupts the protective role of SSTR2 in acute pancreatitis. In the treatment of pancreatitis, we often use somatostatin, however, we are not completely clear about the therapeutic effect and the mechanism. But it is worth affirming that as the half-life of somatostatin is very short, it is not suitable for long-term treatment. On the other hand, somatostatin has a negative effect on the movement of gallbladder Oddi sphincter. Therefore, it has been suggested that doctors should not use secretory drugs to treat acute pancreatitis. Yet, the results from human studies of somatostatin used for therapeutic purposes are not conclusive. Our results provide new insights into the mechanism underlying the controversial therapeutic effects of somatostatin and its analogs in acute pancreatitis treatment.

Acknowledgments

The authors would like to thank all study participants who enrolled in this study.

Funding: This work was supported by the National Basic Research Program of China (973 Program, No. 2012CB822104), National Natural Science Foundation of China (31170766, 81470806), and Nantong City Social Development Projects funds (HS2012032).

Footnote

Reporting Checklist: The authors have completed the ARRIVE reporting checklist. Available at <https://dx.doi.org/10.21037/atm-21-5249>

Data Sharing Statement: Available at <https://dx.doi.org/10.21037/atm-21-5249>

[org/10.21037/atm-21-5249](https://doi.org/10.21037/atm-21-5249)

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at <https://dx.doi.org/10.21037/atm-21-5249>). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The animal study was approved by the Chinese National Committee for the Use of Experimental Animals for Medical Purposes, Jiangsu Branch [JN.No20190930c0480120(233)], and all procedures were carried out following the Guide for the Care and Use of Laboratory Animals (National Research Council, 1996, USA).

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(English Language Editor: B. Draper)

Cite this article as: Huang M, Chen MM, Han D, Chen W, Xu F. N-glycosylation of somatostatin receptor type 2 protects rats from acute pancreatitis. *Ann Transl Med* 2021;9(22):1667. doi: 10.21037/atm-21-5249