



Chinese Pharmaceutical Association
Institute of Materia Medica, Chinese Academy of Medical Sciences

Acta Pharmaceutica Sinica B

www.elsevier.com/locate/apsb
www.sciencedirect.com



ORIGINAL ARTICLE

Neurokinin 1 receptor inhibition alleviated mitochondrial dysfunction *via* restoring purine nucleotide cycle disorder driven by substance P in acute pancreatitis



Chenxia Han^a, Lu Li^a, Lin Bai^b, Yaling Wu^c, Jiawang Li^a,
Yiqin Wang^a, Wanmeng Li^c, Xue Ren^c, Ping Liao^d, Xiaoting Chen^e,
Yaguang Zhang^f, Fengzhi Wu^g, Feng Li^h, Dan Du^{a,c,*}, Qing Xia^{a,*}

^aWest China Center of Excellence for Pancreatitis, Institute of Integrated Traditional Chinese and Western Medicine, West China Hospital, Sichuan University, Chengdu 610041, China

^bHistology and Imaging Platform, Research Core Facility, West China Hospital, Sichuan University, Chengdu 610041, China

^cAdvanced Mass Spectrometry Center, Research Core Facility, Frontiers Science Center for Disease-related Molecular Network, West China Hospital, Sichuan University, Chengdu 610213, China

^dLaboratory of Anesthesia and Critical Care Medicine, National-Local Joint Engineering Research Center of Translational Medicine of Anesthesiology, West China Hospital, Sichuan University, Chengdu 610041, China

^eAnimal Experimental Center, West China Hospital, Sichuan University, Chengdu 610213, China

^fLaboratory of Gastrointestinal Tumor Epigenetics and Genomics, Frontiers Science Center for Disease-related Molecular Network, West China Hospital, Sichuan University, Chengdu 610213, China

^gJournal Center, Beijing University of Chinese Medicine, Beijing 100029, China

^hSchool of Traditional Chinese Medicine, Beijing University of Chinese Medicine, Beijing 100029, China

Received 12 January 2025; received in revised form 1 March 2025; accepted 3 March 2025

KEY WORDS

Acute pancreatitis;
Substance P;
Neurokinin 1 receptor;
 β -Arrestin1;

Abstract Acute pancreatitis (AP) is a life-threatening gastrointestinal disorder for which no effective pharmacological treatments are currently available. One of the pharmacological targets that merits further research is the neurokinin 1 receptor (NK1R), which is found on pancreatic acinar cells and responds to the neuropeptide substance P (SP) that participates in AP. Although a few studies have stated the involvement of SP/NK1R in neurogenic inflammation in AP development, the regulatory mechanism remains unclear. In this study, we found that following activation of NK1R by SP, β -arrestin1, a scaffold protein

*Corresponding authors.

E-mail addresses: xiaqing@medmail.com.cn (Qing Xia), dudan1520@163.com (Dan Du).

Peer review under the responsibility of Chinese Pharmaceutical Association and Institute of Materia Medica, Chinese Academy of Medical Sciences.

<https://doi.org/10.1016/j.apsb.2025.03.037>

2211-3835 © 2025 The Authors. Published by Elsevier B.V. on behalf of Chinese Pharmaceutical Association and Institute of Materia Medica, Chinese Academy of Medical Sciences. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Purine nucleotide cycle;
Mitochondrial
dysfunction;
Magnolol;
Fumarate

of NK1R, down-regulated transcription of *Adss*, *Adsl*, and *Ampd* in the purine nucleotide cycle, thereby inhibiting mitochondrial function through fumarate depletion. Interestingly, we identified magnolol as a new and natural NK1R inhibitor with a non-nitrogenous biphenyl core structure. It exhibited a beneficial effect on AP by restoring purine nucleotide cycle metabolic enzymes and fumarate levels. Our study not only provides new therapeutic strategies, leading compounds, and drug translation possibilities for AP, but also provides important clues for the study of downstream mechanisms driven by SP in other diseases.

© 2025 The Authors. Published by Elsevier B.V. on behalf of Chinese Pharmaceutical Association and Institute of Materia Medica, Chinese Academy of Medical Sciences. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

Acute pancreatitis (AP) is a common digestive disease characterized by severe abdominal pain¹, with mortality up to 20% in severe clinical cases. Currently, there is no targeted drug available^{2,3}. G-protein-coupled receptors are increasingly recognized as promising drug targets in recent studies of AP⁴. The neurokinin 1 receptor (NK1R) is a kind of G-protein-coupled receptor that responds to substance P (SP), a neuropeptide primarily released by peripheral neurons, and mediates multiple diseases, including cancer, central nervous system disorders, and AP^{5,6}. NK1R is located on pancreatic acinar cells and participates in neurogenic inflammation driven by SP in the early AP, but the detailed molecular mechanisms, except for inflammatory pathways such as nuclear factor- κ B^{7,8}, remain unclear. Furthermore, reported NK1R inhibitors mainly consist of peptides and non-peptide nitrogenous molecules, while new chemical entities with high efficiency and low toxicity are still lacking for this target.

β -Arrestin1 is an adapter protein that is essential in the NK1R signaling pathway⁹. Mammals have 2 members of the arrestin family: visual arrestins, which are found only in photoreceptor cells, and non-visual arrestins (β -arrestin1/2), which are universally expressed¹⁰. Once NK1R is activated by the interaction of SP with the extracellular domain of NK1R, cytoplasmic β -arrestin1 is recruited and binds to the C-terminus of NK1R⁹. It aids NK1R internalization and regulates a few intracellular signaling processes following NK1R activation¹¹. Moreover, our previous study observed that SP-induced NK1R internalization in mouse pancreatic acinar cells is accompanied by the translocation of β -arrestin1 into the nucleus¹². Given that β -arrestin1 could regulate the transcription of key genes to modulate cell function under pathological conditions^{13,14}, we propose that the nuclear translocation of β -arrestin1 following SP/NK1R activation in pancreatic acinar cells may induce significant pathological events.

Mitochondria serve as the energy source that maintains the enzymes' secretory function in pancreatic acinar cells¹⁵. Mitochondrial dysfunction leads to adenosine 5'-triphosphate (ATP) depletion and subsequent pancreatic acinar cell death, which represents the major cellular pathology during AP^{16,17}. Purine metabolism is a primary resource for the synthesis of the hub metabolite, inosine monophosphate (IMP), that produces ATP by serial conversions¹⁸. The purine nucleotide cycle is involved in the *de novo* synthesis of IMP and adenosine monophosphate (AMP), catalyzed by 3 metabolic enzymes: adenylosuccinate synthetase (ADSS), adenylosuccinate lyase (ADSL), and AMP deaminase (AMPD). This cycle converts AMP into IMP and reconverts IMP into AMP *via* adenylosuccinate, thereby producing NH₃ and forming fumarate from aspartate. The metabolic flux through the cycle has been proposed to play a role in the regeneration of ATP

and providing tricarboxylic acid cycle intermediates¹⁹. Nevertheless, the role of the purine nucleotide cycle in pathogenesis and its intersection with mitochondrial function during AP have never been reported.

In this study, we first interrogated the target genes and the related pathway that is regulated by β -arrestin1 in the nucleus of primary acinar cells through chromatin immunoprecipitation (ChIP)-sequencing/polymerase chain reaction (PCR). Then, we analyzed the metabolomic data stimulated by SP in pancreatic acinar cells and identified the disordered purine metabolism and tricarboxylic acid cycle. We also determined the damaged mitochondrial morphology, respiration, and membrane potential in pancreatic acinar cells under the stimulation of SP or purine nucleotide cycle inhibitor, as well as their reversal by the NK1R inhibitor CP96345 or fumarate. Lastly, we identified magnolol, a novel class of natural NK1R inhibitor, through click chemistry, molecular interaction, and NK1R knockout experiments to investigate its potential for treating AP. As a result, we have discovered a brand-new mechanism by which the SP-driven NK1R/ β -arrestin1/purine nucleotide cycle axis response impairs mitochondrial activity during AP and provided a new drug candidate for clinical translational research.

2. Methods

Additional information regarding materials and methods for determination of SP in dorsal root ganglion by enzyme-linked immunosorbent assay, isolation of pancreatic acinar cells, isolation of dorsal root ganglion neurons and co-incubation, liquid chromatography-mass spectrometry, gas chromatography-mass spectrometry, real-time quantitative polymerase chain reaction (real-time qPCR), hematoxylin and eosin, immunohistochemistry, chromatin immunoprecipitation (ChIP)-sequencing and ChIP-PCR, cell culture and transfection, targeted metabolomics, ATP detection, cell death assessment, Western blot (WB), reactive oxygen species (ROS) detection, fumarate assessment in pancreatic acinar cells, molecular docking, and cellular thermal shift assay are available in the Supporting Information. The reagents and antibodies are presented in Supporting Information Table S1.

2.1. Animals modeling and drug administration

Beijing Huafukang Biotechnology Co., Ltd. supplied the male C57BL/6J mice (6–8 weeks), which were kept in an environment with a 12-h light–dark cycle, 21 ± 2 °C temperature, standard diet, and unlimited food and water intake. All animal experiments were performed according to the approved protocols of the Animal Ethics Committee of West China Hospital, Sichuan University

(approval No. 2021340A). To induce AP, mice were administered 7 intraperitoneal injections of cerulein (CER) at 1-h intervals, with each injection delivering 50 µg/kg, whereas control (Ctrl) mice received phosphate-buffered saline (PBS) injections. To evaluate the severity, the animals were killed 12 h after receiving their initial CER/PBS injection. For the SP-aggravated CER–AP model, exogenous SP (0.45 mg/kg) was given *via* intraperitoneal injections with the last 3 CER injections. Pancreatic and lung myeloperoxidase (MPO) activity, serum amylase and lipase, and pancreatic trypsin levels were measured following previously published protocols^{12,20,21}. CP96345 (5 mg/kg, TOCRIS, Bristol, UK, CAS Number: 132746-60-2), the NK1R inhibitor, was intraperitoneally injected 30 min before the first CER injection, and magnolol (2.5, 5, and 10 mg/kg) was administered intraperitoneally at the first, fourth, and seventh CER injection for treatment.

2.2. Tissue clearing and immunofluorescent labeling

Pancreatic tissue was cleared, and then immunofluorescent was labeled according to a previous procedure²². In short, the entire mouse pancreas was removed, fixed for 24 h with 10% formalin, and rinsed 5 times with PBS for 30 min, the PBS was removed, the tissue was immersed in CUBIC-L solution (10 g *N*-butyldiethanolamine and 10 g Triton X-100 dissolved in 80 g ddH₂O), and was incubated for 5 days in a 37 °C shaking bed. Following the washing of the tissues using PBS, samples were treated with goat antiserum for 2 days. Subsequently, the primary antibody of anti-neurofilaments 200 (NF200, diluted at a ratio of 1:200) was applied, and the tissues were incubated for 14 days. Afterward, the tissues were washed overnight with PBS, then incubated with a secondary antibody, then the CUBIC-R solution (consisting of 45 g antipyrine and 30 g nicotinamide dissolved in 25 g ddH₂O) was added. Cleared samples were kept at room temperature in black tubes with CUBIC-R before being examined. Pancreas were then imaged using a light sheet 7 microscope (Zeiss, Oberkochen, Germany).

2.3. Adeno-associated virus (AAV) infusion

AAV2/8-(H1-sgRNA.sp(m*Tacr1*))x2-CAG-eGFP-WPRE-pA for pancreatic *Tacr1* knockout (*Tacr1*-KO) and AAV2/8-H1-gRNA(MCS)-CAG-eGFP-WPRE-pA for negative control (NC) were provided by Taitool Biological Co., Ltd. (Shanghai, China). Adenoviruses were administered intraductally using an intraductal infusion system, according to a previously established protocol²³. In brief, mice were administered with a retrograde pancreatic ductal injection of *Tacr1*-KO or NC AAV at a concentration of 1.2×10^{12} genomic copies/mL. Adenoviruses infected mice for 28 days before the establishment of the CER–AP model. The effectiveness of the infection was assessed with real-time quantitative PCR analysis.

2.4. Human sample collection

Human pancreata were collected according to our previous study^{12,21}. Briefly, pancreatic tissues that were used as control were obtained from patients undergoing surgery for left-sided or small unobstructing pancreatic tumors²⁴. AP samples were necrotized, and adjacent inflamed pancreata were resected from AP patients who were suffering from infected pancreatic necrosis during necrosectomy. Major biochemical indexes of patients were given in Supporting Information Table S2. The Institutional

Review Board and Biomedical Ethics Committee of West China Hospital at Sichuan University examined and approved the study protocol (2020, No.196). Patients provided informed consent before sampling.

2.5. Chromatin immunoprecipitation (ChIP)

A cross-linking chemical, formaldehyde, was used to fix mouse pancreatic acinar cells to maintain the DNA–chromatin interaction. To break up and extract the chromatin from DNA, the cells were subjected to sonication. After a specific antibody of β -arrestin1 binding to the chromatin proteins, co-precipitation was carried out by adding protein A/G agarose beads and DNA substrate. To separate the chromatin proteins and DNA substrate, the cross-links between them were broken. Purification of the DNA was performed after protein digestion, which eliminated proteins and contaminants. Thereafter that, the co-precipitated DNA was further analyzed using sequencing or PCR methods.

2.6. Dual luciferase assay

Using Lipofectamine 3000, 200 ng of *Adss*, *Ampd* or *Adsl*, *Arrb1*, and NC plasmids were transfected into HEK293T cells. Cells were lysed with Dual–Glo Luciferase assay reagent 24 h after transfection and measured with a 96-well plate. The firefly luciferase activity was measured by adding 70 µL of Dual–Glo Luciferase test reagent to each well and waiting 10 min for the results to appear on a luminometer. *Renilla* luciferase activity was measured by adding 70 µL of Dual–Glo Stop & Glo test reagent to each well and waiting 10 min for the luminometer to read immediately.

2.7. Oxygen consumption rate (OCR)

The Seahorse XFe24 sensor was hydrated overnight at 37 °C with ambient CO₂. On the day of the experiment, 100 µL of $1 \times$ polylysine was added to the cell plate and incubated for 30 min at 37 °C, then the plate was cleaned with PBS, followed by 400 µL of medium and 100 µL of the cell suspension to each well. After plate calibration, 56 µL of 1.5 µmol/L Olig for the A ports, 62 µL of 0.5 µmol/L FCCP for the B ports, and 69 µL of 0.5 µmol/L RotA for the C ports were added for each well. Then, OCR was automatically calculated by the Seahorse XF-24 analyzer (Agilent, Santa Clara, CA, USA).

2.8. Transmission electron microscope

Freshly isolated mouse pancreatic acinar cells were prefixed in 2.5% glutaraldehyde (pH = 7.4) at 4 °C for 4 h. The samples were then refixed with 1% osmium tetroxide, dehydrated by acetone, and penetrated with a dehydrating agent and Epon-812 embedding agent in a dose-dependent manner with the proportion of 3:1, 1:1, and 1:3. After embedding, samples were prepared into ultra-thin sections and stained with uranyl acetate for 15 min and lead citrate for 2 min. Samples were then observed and photographed by a JEM-1400FLASH transmission electron microscope (JEOL, Tokyo, Japan).

2.9. Mitochondrial depolarization

Mitochondrial depolarization on living pancreatic acinar cells was analyzed according to a previously described protocol²⁵ using the mitochondrial membrane potential dye tetramethylrhodamine.

After 30 min of tetramethylrhodamine (100 nmol/L) incubation in HEPES buffer, pancreatic acinar cells were rinsed with HEPES buffer solution. An Andor Dragonfly 200 high-speed confocal microscope system (Oxford Instruments, Oxfordshire, UK) was used to monitor fluorescence at 594 nm. Image J (National Institutes of Health, Bethesda, MD, USA) was used to analyze the data.

2.10. Bio-layer interferometry analysis

Purified NK1R recombinant protein was provided by HitGen Inc. (Chengdu, China). Briefly, the purified protein was acquired through the manipulation of NK1R's gene expression and fusing sequences of DNA into Sf9 cells. After codon optimization of NK1R, the Bril fusion protein was added in the N-terminal of NK1R (Sequence: S226–Bril–H237, NK1R, 1–335aa; Mutation: E78D, Y121W, Q165A, T222R). Then, the cDNA was cloned in pFastBac1 plasmids and taken up in Sf9 cells. Bac-to-Bac Baculovirus Expression System was used to collect high-titer baculovirus and infect Sf9 cells. Then, the protein was isolated from cells, and protein purification was performed. The purified recombinant NK1R was testified by chromatography and Western blot.

Biolayer interferometry was carried out using an Octet RED96 system (ForteBio Inc., Dallas, TX, USA). NHS–PEG12–Biotin (Thermo Scientific, Waltham, MA, USA) was employed for biotinylation for 1 h according to the manufacturer's instrument, and Desalting Columns (Cytiva, Amersham, UK) were used to remove Tris from the buffer of NK1R and biotin. Streptavidin-coated biosensors (ForteBio Inc.) were wetted in PBS with 0.1% Tween for 10 min before detection. The NK1R recombinant protein (200 µg/µL) was loaded onto the streptavidin-coated biosensors for 10 min. Then, the processes of association and dissociation of magnolol were monitored in parallel. The sensors were transferred into a magnolol protein solution at the 2-fold serial dilutions concentration of 15.6–125 µmol/L. The data were analyzed using the system software Data Analysis 7.0 provided by ForteBio Inc.

2.11. Click chemistry

Conjugated magnolol (cMag) with clickable labels and fluorescent probes was provided by HitGen Inc. (Chengdu, China). The cMag was synthesized *via* sequential formylation of magnolol to generate 5,5'-diallyl-2,2'-dihydroxy-[1,1'-biphenyl]-3-carbaldehyde, conjugation with 2-(prop-2-yn-1-yloxy) ethan-1-amine, and final Borch reaction to generate cMag. Freshly isolated pancreatic acinar cells were added on coverslips that were precoated with the polylysine solution for 30 min. Pancreatic acinar cells were pre-incubated in HEPES buffer with cMag for 10 min. The cells were then treated with CuSO₄ with a designed fluorescent probe. The click reaction mixture was incubated in the dark at room temperature for 10 min. For pancreatic acinar cells treated with CP96345, CP96345 was preincubated for 10 min before cMag incubation. Then, living cells were imaged by a confocal system (Nikon, Tokyo, Japan).

2.12. Statistical analysis

For omics data normalization, the metabolite data were standardized by protein concentration. The ChIP-sequencing data were standardized by determining the peak area of the cover

signal for each IP sample and standardizing it into FPKM signal relative to the standard in a specific region. All data are presented as mean ± standard error of the mean (SEM). Statistical analysis was carried out by GraphPad Prism 8.0.1 software (La Jolla, CA, USA). Unpaired two-tailed Student's *t*-test, one-way ANOVA with Dunnett's multiple comparison tests, or non-parametric tests with the Kruskal–Wallis H test were used for data from two and several groups, respectively. *P*-value <0.05 was considered statistically significant.

3. Results

3.1. AP resulted in neuron firing and SP/NK1R activation triggered β -arrestin1 transcriptional regulation on purine metabolism in pancreatic acinar cells

SP has been massively released by peripheral primary sensory neurons during inflammation²⁶. Although we understand that SP-induced neurogenic inflammation contributes to AP, direct evidence of local pancreatic neuron firing or changes in SP levels during AP is still lacking. We therefore visualized the 3-dimensional neuron innervation in cleared mice pancreas (Fig. 1A). As shown, the pancreatic myelinated A– β fiber neurons labeled by the NF200 antibody in CER-induced AP mice exhibited higher innervation compared to untreated control (Ctrl) mice. This suggests an increased sensitivity of pancreatic sensory neurons induced by CER. The SP levels in dorsal root ganglion and pancreas were both significantly increased in AP compared with Ctrl mice (Fig. 1B). Additionally, the supernatants collected from dorsal root ganglion neurons in AP mice were found to significantly reduce the ATP level in naïve pancreatic acinar cells; this effect was reversed by CP96345, a selective non-peptide NK1R antagonist (Supporting Information Fig. S1A and S1B). Next, we tested the protective effect of pancreatic genetic deletion of NK1R on AP. Pancreatic conditional *Tacr1* (the gene encoding NK1R)-knockout (KO) mice were constructed with AAV-mediated Cas9-*Tacr1* by the pancreatic duct retro-perfusion operation system (Supporting Information Fig. S2A), which resulted in significantly decreased pancreatic *Tacr1* mRNA levels in comparison to the negative control (NC) group (Fig. S2B). Compared with the NC group, pancreatic *Tacr1* knockout significantly decreased AP severity, including serum amylase, lipase (Fig. 1C), and pancreatic histological damage (Fig. 1D). These results indicate that the overactive pancreatic neuron releases SP during AP, and the inhibition of SP/NK1R activation in the pancreas effectively alleviated the injury.

To reveal the pathological events after NK1R activation and β -arrestin1 translocation in the nucleus, we investigated the transcriptional mechanism of β -arrestin1 in the pancreatic acinar cells. To begin with, in human pancreata, we observed a significant increase in the nuclear translocation of β -arrestin1 in AP samples compared to the control group (Fig. 1E). Next, through ChIP experiments, the β -arrestin1-bonded DNA in pancreatic acinar cells from both the Ctrl and AP mice was pulled down and analyzed by sequencing procedure (Fig. 1F). The sites of bonded DNA from Ctrl and AP groups were shown as pie diagrams in Supporting Information Fig. S3A and heatmaps in Fig. 1G. 368 differentially expressed genes were obtained in 2 groups (*P* < 0.05, fold change >2). Based on the Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis of the differential genes, purine metabolism (Fig. 1H) ranked first. The network between the purine

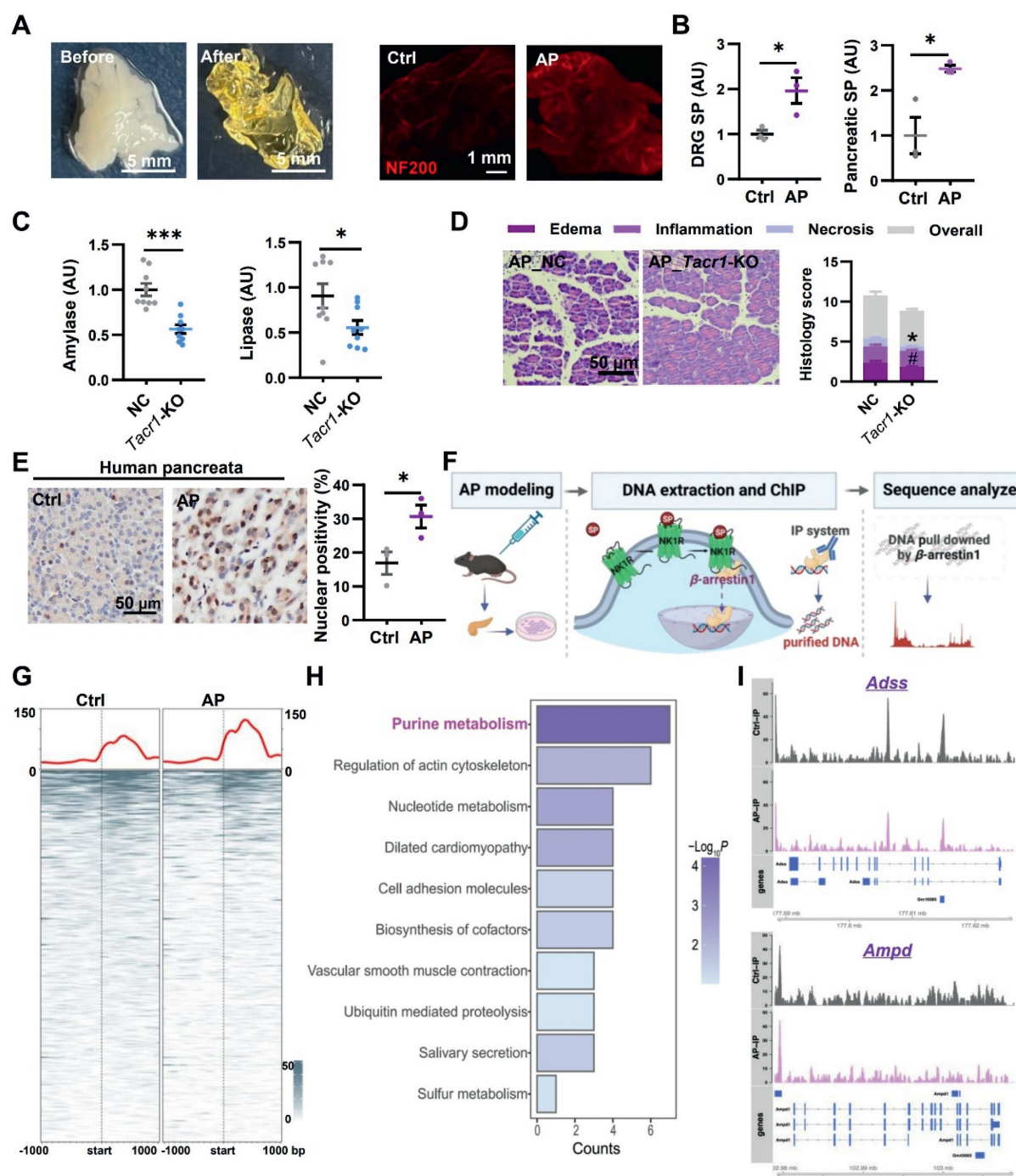


Figure 1 AP resulted in neuron firing and SP/NK1R activation triggered β -arrestin1 transcriptional regulation on purine metabolism in pancreatic acinar cells. (A) Mouse pancreata before and after tissue clearing (left) and representative images of pancreatic neuron innervation after tissue clearing (right) ($n = 3$ mice). (B) SP levels in the dorsal root ganglion (DRG) and pancreas ($n = 3$ mice). (C) Serum amylase and lipase of NC and *Tacr1*-KO mice after AP ($n = 9$ mice). (D) Representative pancreatic histological images and scores of NC and *Tacr1*-KO mice after AP ($n = 8$ mice; * vs. NC for edema, # vs. NC for necrosis). (E) Representative immunohistochemistry images and quantification of β -arrestin1 in the human pancreata ($n = 3$ patients). (F) Schematic diagram of ChIP-seencing ($n = 4$ mice). (G) Heatmap of ChIP-seencing signals across the transcription start site (start, $-1/+1$ kb). (H) Top 10 pathways analyzed by KEGG of differential genes. (I) Integrative genomics viewer plots of β -arrestin1 binding density on *Adss* and *Ampd* in Ctrl and AP groups. Data are presented as mean $\pm$ SEM, * $P < 0.05$, and *** $P < 0.001$ by Student's *t*-test.

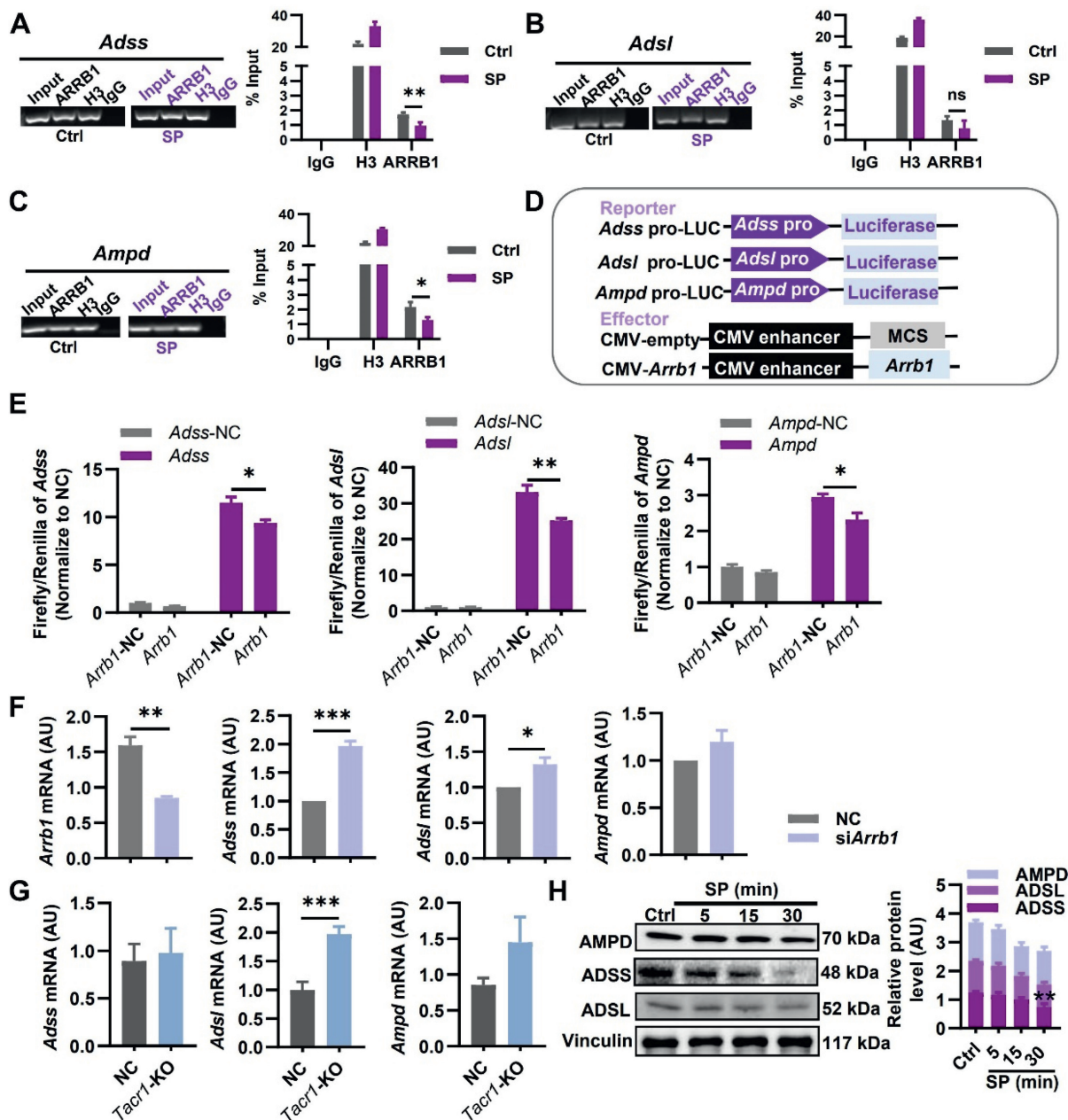


Figure 2 β -Arrestin1-mediated transcriptional inhibition decreased the expression of purine nucleotide cycle metabolic enzymes. Nucleic electrophoresis blot and quantifications of ChIP-PCR for *Adss* (A), *Adsl* (B), and *Ampd* (C) in pancreatic acinar cells. The bar plot labels in gray and purple represent the Ctrl and SP treated groups, respectively ($n = 3$ pooled from 3 mice). (D) The plasmids used in the dual luciferase assays. (E) Activities of firefly luciferase (LUC) and *Renilla* luciferase (REN) were measured and calculated as the LUC/REN ratio and normalized by NC ($n = 4-6$ repeats from 3 independent experiments). (F) The mRNA levels of *Arrb1*, *Adss*, *Adsl*, and *Ampd* after *Arrb1* knockdown in mice pancreatic acinar 266-6 cells ($n = 3$ repeats from 3 independent experiments). (G) The mRNA changes of *Adss*, *Adsl*, and *Ampd* in the pancreas of NC and *Tacr1*-KO mice after AP modeling ($n = 9$). (H) Western blot (WB) images and quantifications of protein changes of ADSS, ADSL, and AMPD under exogenous SP stimulation with different time durations in pancreatic acinar cells ($n = 3$ pooled from 3 mice, ** vs. Ctrl). Data are presented as mean $\pm$ SEM, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; ns, no significance by Student's *t*-test or by one-way ANOVA.

metabolism pathway and its targeted genes is given in Fig. S3B. Among the 7 target genes, *Adss* and *Ampd* represent the key enzymes that operate the purine nucleotide cycle. These 2 genes show decreasing trends after AP modeling according to the peak charts (Fig. 1I). Collectively, these results indicate that β -arrestin1 participated in purine metabolism by transcriptionally regulating enzymes involved in the purine nucleotide cycle during AP.

3.2. β -Arrestin1-mediated transcriptional inhibition decreased the expression of purine nucleotide cycle metabolic enzymes

ChIP-PCR was further performed to test the levels of β -arrestin1-bonded *Adss*, *Adsl*, and *Ampd* in untreated (Ctrl) and SP-treated primary pancreatic acinar cells of mice. In comparison to the Ctrl group, significant decreases in mRNA levels for both *Adss* (Fig. 2A)

and *Ampd* (Fig. 2C), and a downward trend without significance in *Adsl* gene level (Fig. 2B) after SP treatment of primary pancreatic acinar cells were observed. This result suggests that SP emerged down transcription of *Adss* and *Ampd* via β -arrestin1. To provide direct evidence for the effect of β -arrestin1 on gene transcription of purine nucleotide cycle metabolic enzymes, we conducted a dual luciferase assay using HEK293T cells transfected with the corresponding reporter of *Adss*, *Adsl*, and *Ampd* plasmids (Fig. 2D). It was found that overexpression of *Arrb1* (the gene encoding β -arrestin1) resulted in significantly decreased transcriptional activities of promoters for *Adss*, *Adsl*, and *Ampd* (Fig. 2E). Correspondingly, in mice pancreatic acinar cancer cell lines 266-6, under stimulation of SP, knockdown of *Arrb1* significantly increased these genes' expression (Fig. 2F). Increasing mRNA levels of purine nucleotide cycle metabolic enzymes were also observed in the pancreas of *Tacr1* knockout mice (Fig. 2G). In freshly isolated mouse pancreatic acinar cells, we found that SP treatment significantly reduced protein (Fig. 2H) expressions of ADSS at 30 min, and ADSL and AMPD without significance, as well as their mRNA levels (Supporting Information Fig. S4). Thus, SP/NK1R triggers β -arrestin1 to inhibit the transcription of purine nucleotide cycle metabolic enzymes during AP.

3.3. SP/NK1R activation resulted in mitochondrial dysfunction and ATP depletion in pancreatic acinar cells

To explain the cellular function that is affected by SP/NK1R activation, a targeted metabolomic analysis was performed on freshly isolated pancreatic acinar cells according to a previous study²⁷. 112 metabolites were determined in both Ctrl and SP-treated groups, and the SP-treated group clustered apart from the Ctrl group in the first 2 principal component projections (Fig. 3A). A total of 19 metabolites (Fig. 3B) with significant differences were filtered out ($P < 0.05$, fold change >1.3 , or < 0.78). Notably, SP treatment resulted in significant decreases in several metabolites involved in purine salvage synthesis, including adenosine, inosine, hypoxanthine, guanosine 5'-monophosphate (GMP), and AMP. The changes of representative purines were given in Fig. 3C. Further, KEGG enrichment of differential metabolites suggested that purine metabolism was the most impacted metabolic pathway, which was agreed with the ChIP-sequencing results. Notably, the tricarboxylic acid cycle was also found to be affected obviously (Fig. 3D and Supporting Information Fig. S5). Mitochondria have a critical role in the production of intracellular ATP and participate in the generation of intermediates necessary for the biosynthesis of purines by use of tricarboxylic acid cycle metabolites²⁸. Mitochondrial dysfunction and ATP depletion are considered to be the fundamental mechanisms of pancreatic acinar cell injury in AP^{16,17}. As expected, the inhibition of NK1R by CP96345 significantly rescued SP-induced mitochondrial maximum respiration (Fig. 3E) and mitochondrial morphological changes, including swelling, deforming, and crest breaking (Fig. 3F). Furthermore, CP96345 significantly improved mitochondrial depolarization (Fig. 3G and H) and reactive oxygen species (ROS) generation induced by SP (Fig. 3I and J). Given that the ATP depletion was found in a time- and dose-dependent manner in pancreatic acinar cells stimulated by SP (Supporting Information Fig. S6), we also identified that the NK1R inhibitor significantly restored the level of ATP and partly restored AMP levels in pancreatic acinar cells (Fig. 3K), as well as protected against cell necrosis (Supporting Information Fig. S7). Thus, these results together suggest that SP/NK1R activation

induced mitochondrial dysfunction and ATP depletion in pancreatic acinar cells (Fig. 3L).

3.4. Purine nucleotide cycle inhibition led to mitochondrial dysfunction in pancreatic acinar cells attributed to the concomitant fumarate consumption

Next, we investigated whether the mitochondrial dysfunction and ATP depletion induced by NK1R activation are caused by abnormal purine metabolism, especially purine nucleotide cycle disorder. The purine nucleotide cycle was recognized to have an intersection with mitochondrial function; this process mainly lies in the concomitant production of fumarate by ADSL to feed the tricarboxylic acid cycle²⁹ (Fig. 4A). Alanosine, which acts as an inhibitor of ADSS³⁰, the rate-limiting enzyme in the purine nucleotide cycle, is capable of inducing cell necrosis (Supporting Information Fig. S8A) and a reduction in intracellular ATP (Fig. S8B). We first detected fumarate levels in SP- and alanosine-treated pancreatic acinar cells, respectively, and the results suggested that both SP and alanosine significantly decreased cellular fumarate levels (Fig. 4B). Notably, CP96345 did not change alanosine-mediated fumarate loss (Supporting Information Fig. S9), but successfully increased SP-induced fumarate loss (Supporting Information Fig. S10), suggesting that fumarate loss after NK1R activation was mainly mediated by purine nucleotide cycle inhibition. In addition, overexpression of *ADSL* and *AMPD* both elevated the cellular fumarate levels in HEK293T cells (Supporting Information Fig. S11). Then, the rescuing effect of fumarate against mitochondrial dysfunction by SP stimulation and alanosine treatment was examined on pancreatic acinar cells. Fumarate protected pancreatic acinar cells from the decreased mitochondrial respiration induced by both SP (Fig. 4C) and alanosine (Fig. 4D). Most importantly, fumarate restored the cellular ATP levels that were reduced by these 2 toxins (Fig. 4E). Thereafter, we evaluated mitochondrial morphological changes, mitochondrial depolarization, and ROS generation induced by alanosine and subsequently restoration by fumarate. As shown, fumarate significantly improved alanosine-induced mitochondrial morphological changes, including swelling and crest breaking (Fig. 4F), as well as mitochondrial depolarization (Fig. 4G and H) and ROS generation (Fig. 4I and J). Taken together, the above results indicated that the inhibition of the purine nucleotide cycle directly leads to fumarate deficiency, which subsequently resulted in mitochondrial dysfunction, while supplementation of fumarate successfully reversed these changes.

3.5. Magnolol protected from SP-induced mitochondrial injury by inhibition of NK1R on pancreatic acinar cells

Existing chemical inhibitors of NK1R are predominantly designed based on the nitrogen-containing scaffold of CP96345 and typically feature a benzyl- or phenyl-substituted *N*-heterocycle core structure³¹ (Supporting Information Fig. S12). They showed less satisfaction in conditions like analgesia and are often accompanied by multiple adverse effects^{32,33}. Therefore, it is necessary to provide a highly efficient and low-toxicity NK1R inhibitor with a novel skeleton. Here, we focus on magnolol, a non-nitrogenous natural product belonging to the lignan family, which has been reported to be one of the quality markers of Chaiqin chengqi decoction used for treating AP and alleviating pain^{12,34}. We examined whether magnolol could target NK1R to ameliorate mitochondrial dysfunction of pancreatic acinar cells and AP. By

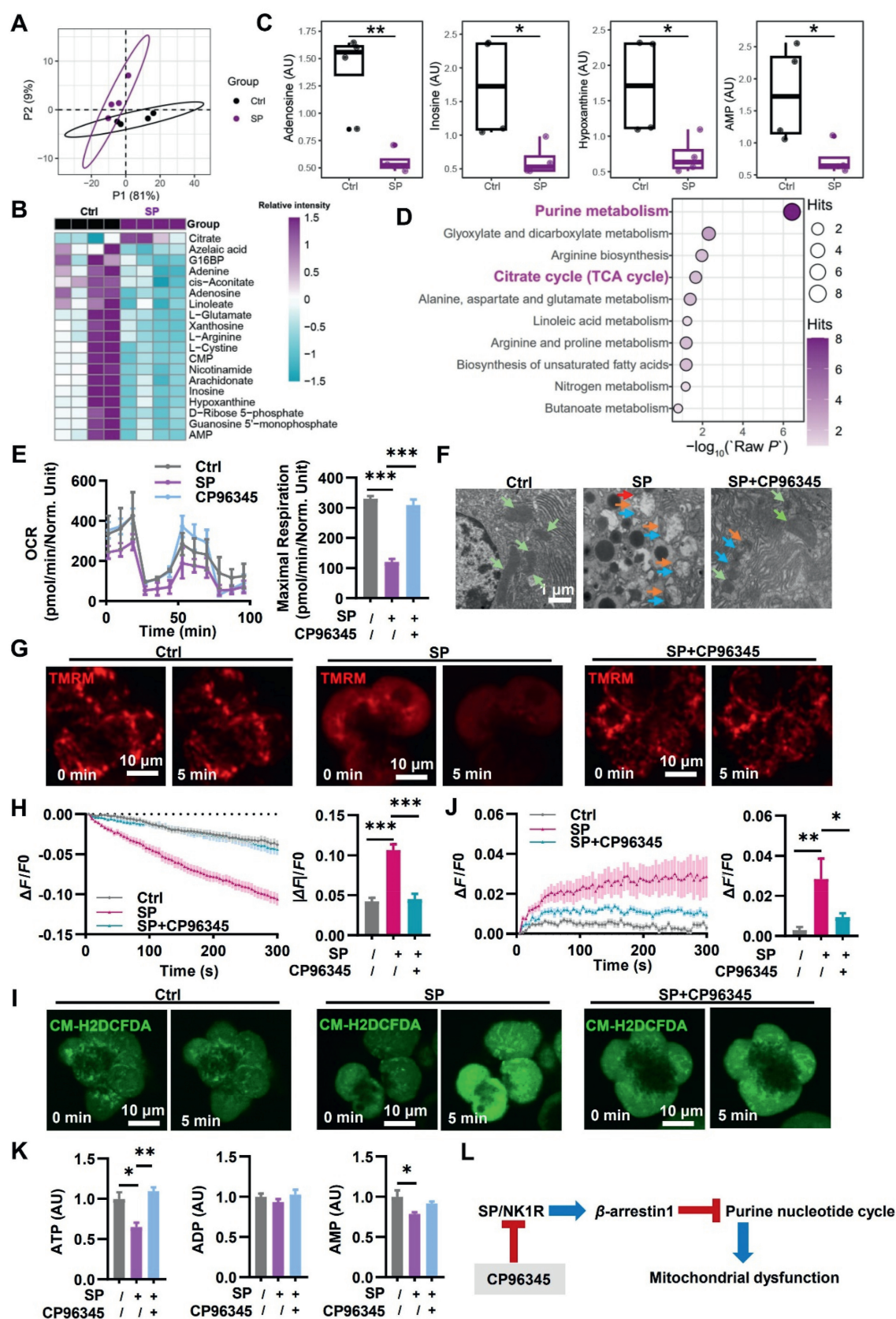


Figure 3 SP/NK1R activation resulted in mitochondrial dysfunction and ATP depletion in pancreatic acinar cells. (A) Partial least-squares discriminant analysis score plot of Ctrl and SP-treated pancreatic acinar cells ($n = 4$ pooled from 4 mice). (B) Heat map of metabolites with significant difference between Ctrl and SP-treated pancreatic acinar cells ($n = 4$ pooled from 4 mice). (C) Significantly changed purines between

molecular docking and cellular thermal shift assay, we found that magnolol significantly stabilized NK1R protein under 70 °C and 75 °C in both Ctrl and SP-stimulated cell lysis in pancreatic acinar cells (Fig. 5A). This result was confirmed by a small molecule interaction assay, and the data indicated that magnolol could interact with recombinant NK1R protein with high affinity ($K_D = 89 \mu\text{mol/L}$) (Fig. 5B). To provide solid evidence supporting the physical interaction between magnolol and NK1R, we designed a small molecular probe of magnolol (Fig. 5C), termed conjugated-magnolol (cMag). The synthesis of cMag began with the conversion of magnolol to 5,5'-diallyl-2,2'-dihydroxy-[1,1'-biphenyl]-3-carbaldehyde (**1**) via a formylation reaction. Then, (**1**) was treated with 2-(prop-2-yn-1-yloxy) ethan-1-amine, followed by a Borch reaction, and generated cMag (Supporting Information Fig. S13). Then the cMag can be conjugated with an azide-containing fluorophore via the alkyne bond under Cu^{2+} catalysis, leading to the red fluorescence emission (Fig. 5C). Furthermore, through molecular docking analysis, hydrogen bonding interactions were identified between cMag and the binding pocket in the substrate-binding domain of NK1R (PDB:6HLO) (Supporting Information Fig. S14). These interactions confirmed the binding of cMag to NK1R, which is similar to magnolol (Fig. 5D). The comparable bioactivity of cMag to magnolol was evidenced by its protection against taur-olthocholic acid sulfate (TLCS)-induced cell death (Fig. 5E and F) and ATP reduction (Supporting Information Fig. S15). Next, competitive inhibition of NK1R by cMag or CP96345 was performed on living pancreatic acinar cells. As shown in Fig. 5G, after the click chemical reaction, cMag with red fluorescence was visualized on the plasma membrane of living pancreatic acinar cells, and this fluorescent signal was significantly decreased in the case of CP96345 pre-incubation (Fig. 5H). This result indicated the physical interaction of magnolol and NK1R. Additionally, *Tacr1* knockdown significantly increased cellular ATP levels in pancreatic acinar cell lines 266-6 after SP stimulation, and the beneficial effect was not affected by magnolol (Supporting Information Fig. S16). These results suggest that magnolol could improve AP by directly inhibiting NK1R in pancreatic acinar cells. Next, we examined whether magnolol could restore purine nucleotide cycle metabolic enzyme expressions and mitochondrial dysfunction damaged by SP on pancreatic acinar cells. As shown in Fig. 5I, magnolol could restore the protein levels of ADSS, ADSL, and AMPD. Further, magnolol significantly improved cellular fumarate (Fig. S11), mitochondrial respiration (Fig. 5J), and morphological changes (Fig. 5K). Hence, magnolol protected against SP-induced mitochondrial injury by inhibition of NK1R on pancreatic acinar cells.

Finally, the protective effects of magnolol on the SP-aggravated CER-AP mouse model were investigated. Magnolol

was administered at doses of 2.5, 5, and 10 mg/kg, respectively, while CP96345 served as a positive control. As expected, 5 and 10 mg/kg of magnolol significantly decreased serum amylase and lipase compared with the AP group (Fig. 6A). Meanwhile, 2.5 and 5 mg/kg of magnolol significantly reduced pancreatic MPO, which represents the neutrophil infiltration, while magnolol at 10 mg/kg significantly reduced lung MPO, indicating the protective effect against AP-induced lung injury compared with the AP group (Fig. 6A). All doses of magnolol improved the overall scores of pancreatic histological damages (Fig. 6B and C). However, no significances were observed between the magnolol groups and the CP96345 group in these parameters. These data supported that magnolol could protect AP from local and systematic injury. Additionally, we evaluated the protective effects of magnolol using pancreatic conditional *Tacr1*-knockout AP mice, and there was no significant difference between the magnolol-treated and untreated groups in the AP severity parameters (Fig. 6D and E). Therefore, the *in vivo* protective effects of magnolol against AP are mainly mediated through NK1R.

Collectively, we found that after SP/NK1R activation in pancreatic acinar cells, β -arrestin1 emerged transcriptional inhibition on *Adss*, *Adsl*, and *Ampd*, which led to purine nucleotide cycle impairment. This disorder directly induced fumarate consumption and resulted in mitochondrial dysfunction. Magnolol could successfully decrease these cellular pathologies by inhibitive targeting NK1R (Fig. 7).

4. Discussion

Despite prior studies investigating the role of SP/NK1R in activating inflammatory signaling pathways such as mitogen-activated protein kinases and nuclear factor- κB ^{7,35,36} in AP, the central mechanism related to early pancreatic acinar cell injury driven by SP/NK1R remains to be fully elucidated. Here, we have provided convincing evidence suggesting that activation of SP/NK1R led to the suppression of the purine nucleotide cycle regulated by β -arrestin1. This suppression ultimately results in the consumption of fumarate and leads to mitochondrial dysfunction. We have also identified magnolol, a novel and natural NK1R inhibitor, as a potential drug candidate for the treatment of AP. This is the first report on the precise mechanism of neural regulation on metabolism in acinar cells, which paves the way for translational research into the inhibition or neutralization of toxic neurotransmitters in early AP.

The pancreas is innervated by enormous peripheral neurons, which organize the endo- and exocrine functions of the pancreas^{37,38}. During occurrences of AP, peripheral neurons become excessively active and release neurotransmitters that

Ctrl and SP treated pancreatic acinar cells ($n = 4$ pooled from 4 mice). (D) The top 10 impacted pathways based on KEGG analysis of differential metabolites. (E) The determination of OCR in pancreatic acinar cells ($n = 3$ pooled from 3 mice). (F) Representative electron microscopic images of the mitochondria in pancreatic acinar cells (green arrows represent normal mitochondria, blue arrows represent swelling, orange arrows represent crest broken, and red arrows represent deforming changes of mitochondria). Representative images (G), traces, and quantifications (H) of mitochondrial depolarization measured by Tetramethylrhodamine (TMRM) during living-cell imaging of pancreatic acinar cells ($n = 23$ –28 cells pooled from 3 mice). Representative images (I), traces, and quantifications (J) of ROS generation during living-cell imaging of pancreatic acinar cells ($n = 9$ cells pooled from 3 mice). (K) ATP, adenosine diphosphate (ADP), and AMP levels in pancreatic acinar cells were analyzed by LC–MS/MS ($n = 3$ pooled from 3 mice). (L) Schematic diagram illustrating relationships among SP/NK1R, β -arrestin1, purine nucleotide cycle, and mitochondrial dysfunction (blue arrows with solid lines represent activation, red lines with rods represent inhibition). Data are presented as mean $\pm$ SEM, * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ by Student's *t*-test or by one-way ANOVA.

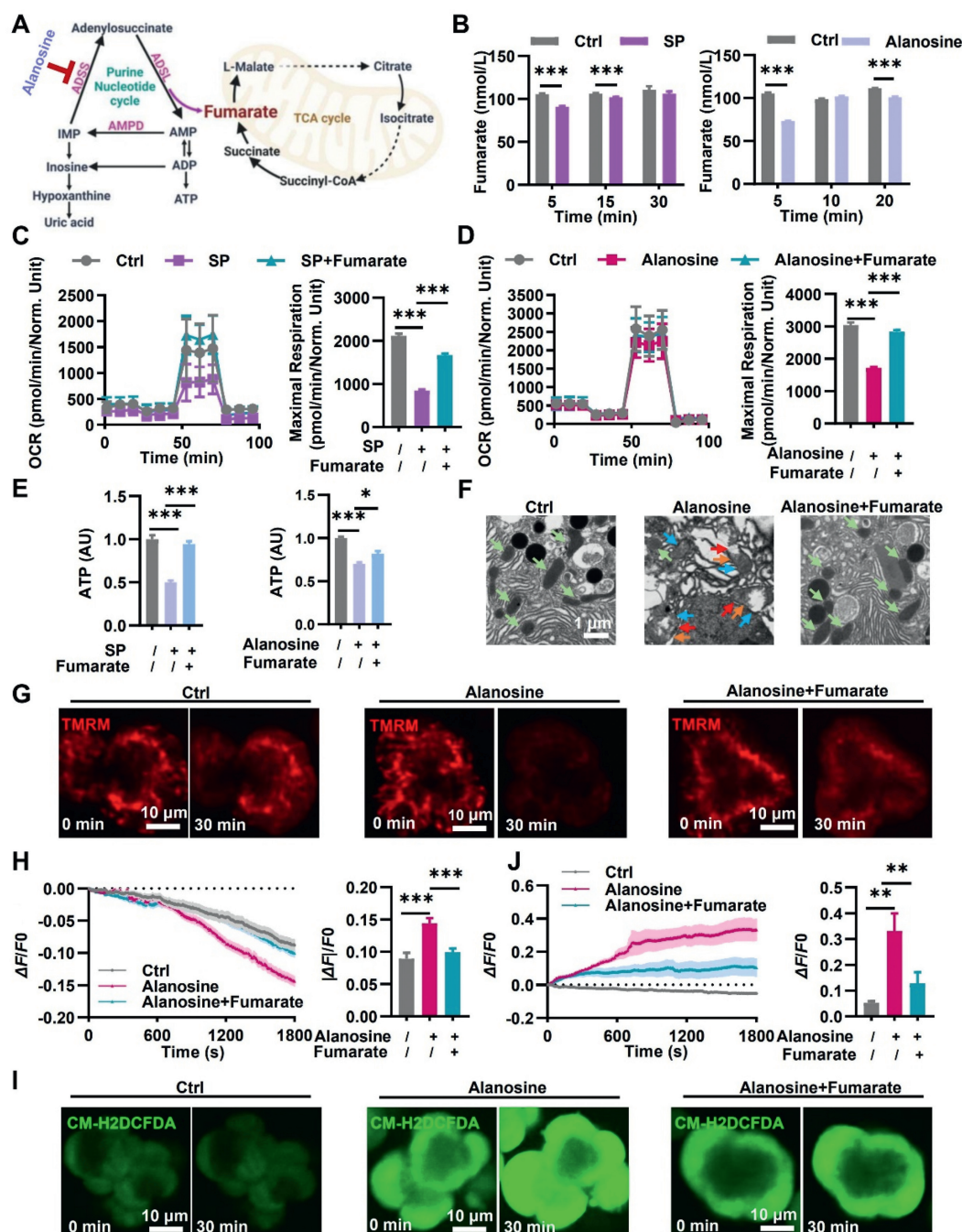


Figure 4 Purine nucleotide cycle inhibition led to mitochondrial dysfunction in pancreatic acinar cells attributed to the concomitant fumarate consumption. (A) The schematic diagram of the relationship between the purine nucleotide cycle and tricarboxylic acid cycle (TCA). (B) Fumarate levels in pancreatic acinar cells under SP and alanosine stimulations with different time durations ($n = 3$ repeats pooled from 3 mice). Protective effects of fumarate against SP- (C) and alanosine- (D) induced OCR in pancreatic acinar cells ($n = 3$ repeats pooled from 3 mice). (E) Protective effects of fumarate on cellular ATP during SP and alanosine stimulations of pancreatic acinar cells ($n = 3$ repeats pooled from 3 mice). (F) Representative electron microscopic images of mitochondria in pancreatic acinar cells (green arrows represent normal mitochondria, blue arrows represent swelling, and orange arrows represent crest broken, and red arrows represent deforming changes of mitochondria). Representative images (G), traces and quantifications (H) of mitochondrial depolarization measured by TMRM during living-cell imaging of pancreatic acinar cells ($n = 33$ – 36 cells pooled from 3 mice). Representative images (I), traces, and quantifications (J) of ROS generation during living-cell imaging of pancreatic acinar cells ($n = 5$ cells pooled from 3 mice). Data are presented as mean $\pm$ SEM, $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$ by Student's t -test or by one-way ANOVA.

promote local inflammation^{39,40}. It has been recognized that the elevated axonal myelination is related to peripheral neuron firing⁴¹. As a classic neurotransmitter mediating pain and inflammation, SP has been massively reported to play a central role in various disease^{5,42}. Previous studies have highlighted the role of SP as an exogenous toxin in inducing cellular damage and activating inflammatory pathways in AP⁴³; however, evidence regarding its origin from neuron cells remains inconclusive. Here, based on tissue clearance, we visualized the changes of neuron axon myelination in the pancreas and observed hyperactivation of pancreatic innervated sensory neurons during AP. The simultaneous detection of SP levels in both dorsal root ganglion and the pancreas indicates the elevations of SP in both the cell body and axon terminals of peripheral neurons during AP. Additionally, the supernatant of the dorsal root ganglion neuron cells is capable of damaging acinar cells by reducing their ATP generation. These findings together confirmed that the neuron-originated SP played an essential role in injuring pancreatic acinar cells.

Next, we intended to investigate the cellular process driven by SP. NK1R is the predominant receptor of SP with the highest affinity that is widely distributed on pancreatic acinar cells^{9,44}. The current study confirmed that pancreatic deletion of *Tacr1* emerged protective effects on the AP experimental model. NK1R functions by associating with the adapter protein β -arrestin1⁹. Due to the possibility of the translocation of β -arrestin1 into the nucleus of pancreatic acinar cells¹², we proceeded to reveal its role in transcriptional regulation in AP by ChIP-sequencing/PCR of isolated pancreatic acinar cells. Accordingly, we observed the transcriptional downregulation of the *Adss*, *Adsl*, and *Ampd* genes in the purine nucleotide cycle mediated by β -arrestin1. The relationship between β -arrestin1 and purine nucleotide cycle enzyme was further confirmed by the dual luciferase technique and gene deletion experiments in HEK293T cells or 266-6 cell lines. While β -arrestin1 has been previously reported to play key roles in regulating gene transcription, such as p27, c-fos¹³, hTERT⁴⁵, and mediating histone H4 acetylation of BCR/ABL⁴⁶, there is currently no evidence that it affects the transcription of metabolic enzymes. Therefore, our findings suggested a potential pathophysiology of NK1R activation that causes β -arrestin1 to enter the nucleus and regulate the transcription of purine nucleotide cycle metabolic enzymes, as well as providing a possible avenue for further research motivated by SP.

The *de novo* purine synthesis initiates with 5-phosphoribosyl-1-pyrophosphate and proceeds through 10 steps to generate IMP. IMP serves as a central hub metabolite for the synthesis of AMP and GMP. The purine nucleotide cycle is involved in the *de novo* synthesis of IMP and AMP¹⁹. Increased purine metabolism was observed in diabetic AP mice, and excessive IMP stimulates granulocyte production and accelerates disease progression⁴⁷. AMP is an important regulator for AMP-activated protein kinase, which is involved in alleviating pancreas and organ injury in AP progression^{48,49}. In our study, we observed decreased protein expression of ADSS, ADSL, and AMPD in the purine nucleotide cycle, as well as reduced levels of purine metabolites such as AMP, inosine, adenosine, and hypoxanthine in SP-stimulated primary acinar cells. These results suggest that purine metabolism, especially the purine nucleotide cycle, is inhibited, which may eventually affect ATP production and purine-activated

signaling pathways. Depleted ATP levels and increased cell necrosis were observed in both the SP-treated and ADSS inhibitor-treated groups, which are consistent with prior work that inhibiting the purine nucleotide cycle results in decreased proliferation of hepatocellular carcinoma cells⁵⁰. Hence, the purine nucleotide cycle plays an essential role in maintaining cellular energy and viability in AP development.

Mitochondria have a critical role in the production of intracellular ATP and participate in the generation of intermediates necessary for the biosynthesis of purines²⁹. Mitochondrial dysfunction is a hallmark of early cellular pathology in AP¹⁶. Our subsequent investigation focused on whether purine nucleotide cycle inhibition has an intersection with mitochondrial dysfunction. In addition to providing purine substrates for ATP synthesis, the purine nucleotide cycle can also promote mitochondrial biosynthesis⁴⁷ and activity⁵¹. Among these, it is worth noting that fumarate, generated through purine nucleotide cycle metabolic flux, serves as an intermediate in the tricarboxylic acid cycle in mitochondria^{52,53}. A previous report indicated that the purine nucleotide cycle-supplied fumarate regulates mitochondrial activity in hepatocellular carcinoma cells by maintaining the balance of mitochondrial electron transport⁵⁴. In our study, a reduction in fumarate levels was observed in both SP-treated and ADSS inhibitor-treated cells, while an elevation was found in cells overexpressing *ADSL* and *AMPD* genes. Furthermore, both the inhibition of NK1R and the supplementation of fumarate restored mitochondrial dysfunction in pancreatic acinar cells. The above results further confirm the protective effect of fumarate on AP⁵⁵, and emphasize its significance in the connection between the purine nucleotide cycle and mitochondrial function.

The existing NK1R inhibitors, including peptides and non-peptide nitrogenous molecules, are mostly designed based on CP96345⁵⁶, which is characterized by the presence of an *N*-heterocyclic quinuclidine core. The successful clinical application of NK1R inhibitors has been demonstrated in the management of chemotherapy-induced nausea and vomiting^{32,33}. Due to the side effects such as sedation and motor impairment⁵⁷, there remains a critical need for the development of efficient and low-toxicity structural scaffolds that inhibit NK1R. Magnolol is an active lignan from *Chaikin chengqi* decoction used for AP intervention in clinic^{58,59}. In our previous study¹², it was indicated that magnolol reduced SP-induced NK1R internalization, but whether magnolol can act as an effective NK1R inhibitor remains unclear. Here, based on molecular docking between magnolol and NK1R, the hydrogen bond, π - π stacking, and hydrophobic interactions suggest that magnolol could bind to NK1R. Further cellular thermal shift assays, bio-layer interferometry analysis, and click chemistry experiments *via* living-cell imaging all confirmed the physical interaction between magnolol and NK1R. Together with the ineffective response of magnolol on *Tacr1* deletion *in vitro* and *in vivo*, it was implied that magnolol serves as an effective NK1R inhibitor that alleviates AP severity. Meanwhile, owing to the hypotoxicity, high activity and low cost of magnolol⁶⁰, as well as its various nano-formulations, including mixed micelles⁶¹, emulsions⁶², and nanoparticles⁶³, which help optimize the water solubility and bioavailability, this natural product has the potential for future clinical translational research in AP and other diseases.

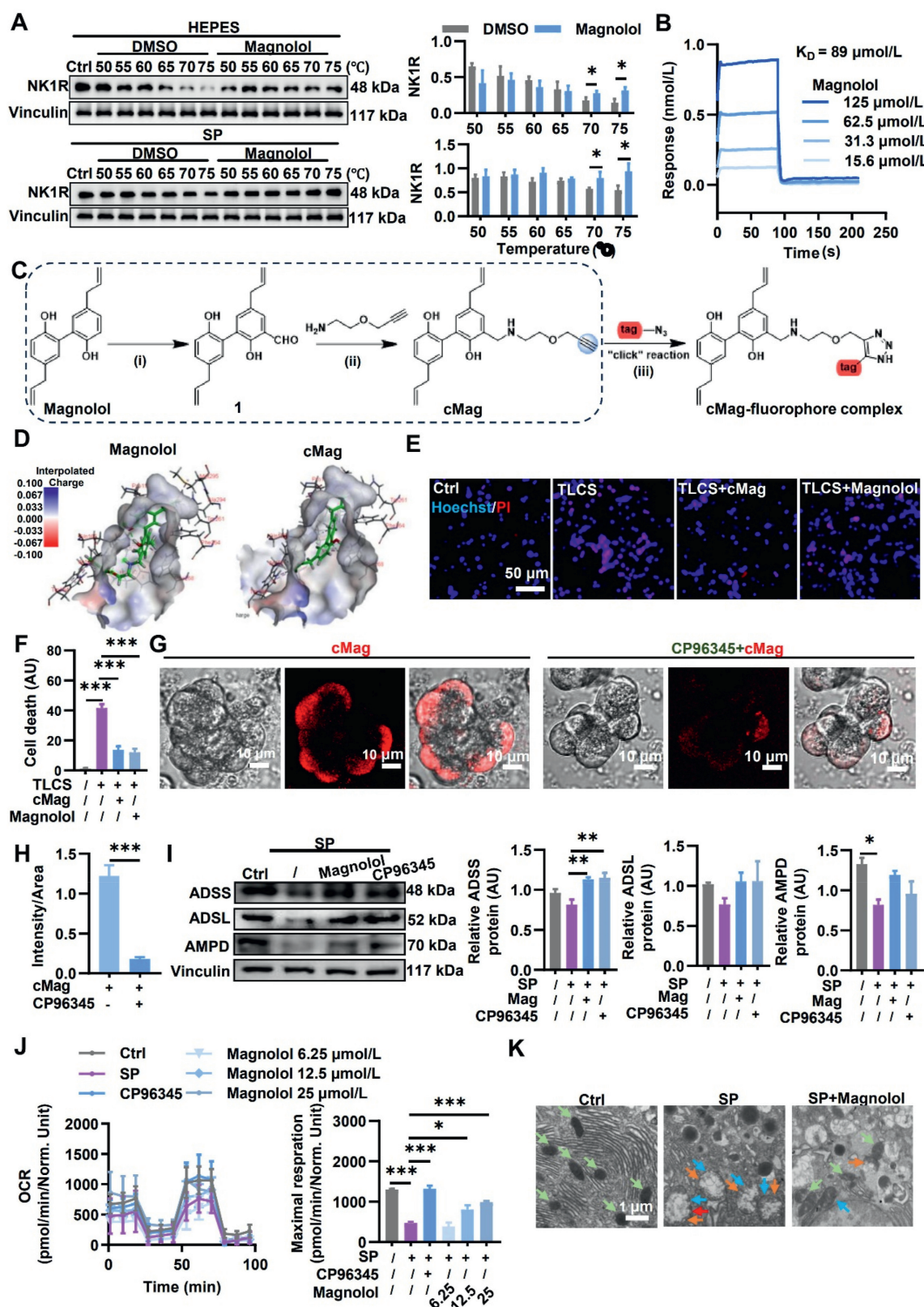
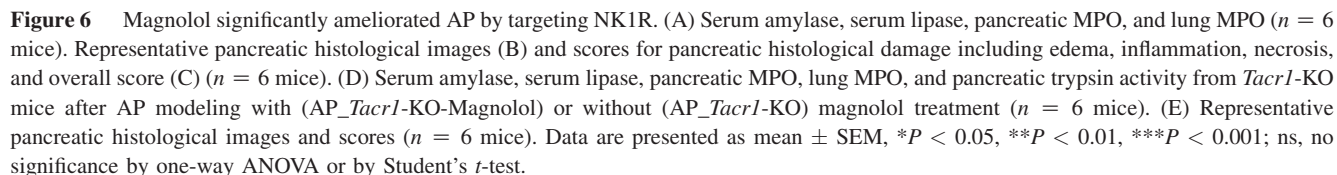


Figure 5 Magnolol protected against SP-induced mitochondrial injury by inhibition of NK1R on pancreatic acinar cells. (A) Effect of magnolol on thermal stability of NK1R in pancreatic acinar cells ($n = 3$ repeats pooled from 3 mice). (B) Bio-layer interferometry analysis of magnolol binding to NK1R recombinant protein ($K_D = 89 \mu\text{mol/L}$). (C) Synthesis workflow indicating the designed clickable cMag with an alkyne, reacting with a fluorophore tagged by an azide under the catalyzation of Cu^{2+} to form the cMag-fluorophore complex. Reaction conditions: (i) $(\text{CH}_2\text{O})_n$ (4.0 eq.), MgCl_2 (3.0 eq.), Et_3N (1.0 eq.), 60°C , overnight; (ii) NaCNBH_3 (1.7 + 1.7 eq.), AcOH (1.0 eq.), MeOH , 25°C , 6 h. (iii)



CuSO₄, room temperature, 10 min. (D) Molecular docking of magnolol/cMag binding to NK1R. Representative images (E) and quantitative results (F) of the protective effect of cMag/magnolol on cell death of pancreatic acinar cells measured by PI staining ($n = 3$ repeats pooled from 3 mice). Confocal fluorescent images (G) and quantitative results for fluorescent intensity (H) of cMag in living pancreatic acinar cells after click chemistry ($n = 7$ cells pooled from 3 mice). (I) The protein level of ADSS, ADSL, and AMPD was modulated by magnolol after SP stimulation on pancreatic acinar cells ($n = 3$ repeats pooled from 3 mice). (J) Protective effect of magnolol on OCR of pancreatic acinar cells ($n = 3$ repeats pooled from 3 mice). (K) Representative electron microscopic images of mitochondria of pancreatic acinar cells (green arrows represent normal mitochondria, blue arrows represent swelling, orange arrows represent crest broken, and red arrows represent deforming changes of mitochondria). Data are presented as mean $\pm$ SEM, * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ by Student's t -test or by one-way ANOVA.

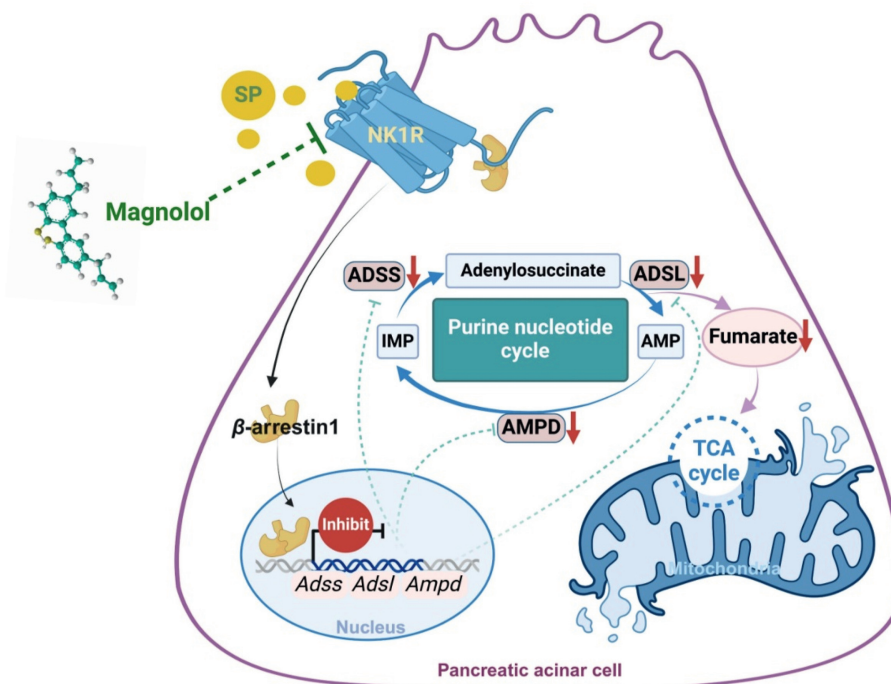


Figure 7 Cellular mechanism for SP/NK1R/ β -arrestin1 mediated purine nucleotide cycle inhibition and mitochondrial dysfunction. During AP, SP was released and bound with NK1R, then β -arrestin1 is activated and transferred into nucleus to emerge transcriptional inhibition effect on *Adss*, *Adsl*, and *Ampd*. The inhibition of these enzymes leads to impaired PNC, and subsequently induces fumarate consumption, which further induces tricarboxylic acid (TCA) cycle breakdown and mitochondrial dysfunction. Magnolol could protect against pancreatic cell injury by interacting with NK1R. (Solid lines with arrows denote known knowledge, while dotted lines with arrows or rods indicate unknown information.)

5. Conclusions

In summary, we demonstrated the cellular mechanisms of the SP/NK1R– β -arrestin1 axis in contributing to purine nucleotide cycle disorder by transcriptionally inhibiting the enzymes catalyzing this cycle. We also established a connection between purine nucleotide cycle inhibition and mitochondrial dysfunction through the hub metabolite fumarate. Additionally, we identified magnolol as the first non-nitrogenous NK1R inhibitor in AP treatment. Our study first elucidated the coordinated mechanism that involves the excessive stimulation of neurons and the disruption of metabolism following G-protein-coupled receptor signaling. The new biphenyl-structural natural NK1R antagonist offers valuable candidates for future drug discovery aimed at targeting NK1R for enhanced clinical use. We note 2 limitations in our study. Firstly, it has been known that β -arrestin1 remains active in the cytoplasm after dissociation from G-protein-coupled receptors⁶⁴. However, it remains unclear how distant β -arrestin1 (or dissociated β -arrestin1 from NK1R) in the cytoplasm translocates into the nucleus in pancreatic acinar cells. Secondly, it is possible that other linkages between NK1R activation and purine nucleotide cycle disorder exist, except β -arrestin1, which needs further research.

Acknowledgments

The study was supported by the National Natural Science Foundation of China (No. 82104598 to Chenxia Han, No. 82274321 to Qing Xia, No. 82170905 to Dan Du), Project of Sichuan Provincial Administration of Traditional Chinese Medicine (2023ZD04

to Qing Xia, China). We thank Li Li, Chunjuan Bao, and Fei Chen (Institute of Clinical Pathology, West China Hospital) for their technical assistance with the immunohistochemistry and hematoxylin and eosin staining. We thank Manjiangcuo Wang, Qianlun Pu, Rui Wang, Na Jiang, Fei Fu, and Yijing Long (Mass Spectrometry Center, West China Hospital) for helping with metabolomic sample preparation and analysis. We thank Yaping Wu and Qiuxiao Shi (Histology and Imaging Platform, West China Hospital) for helping with the tissue clearing and imaging. We thank Maodi Xie (Laboratory of Mitochondria and Metabolism, West China Hospital) for helping with the Seahorse experiment. We thank Sisi Wu, Yanjing Zhang, Yinchuan Wang, Jinkui Pi, Yan Wang, and Liqiang Hu (Research Core Facility, West China Hospital) for helping with biomolecular experiments. We thank Yun Ma and Xinran Zhou (Biobank, West China Hospital) for supporting the human sample collection.

Author contributions

Qing Xia and Dan Du designed the study. Chenxia Han and Lu Li conceived the *in vitro* experiments. Yaling Wu and Xue Ren helped with the probe design and molecular docking. Chenxia Han, Lu Li, and Jiawang Li conducted *in vivo* experiments. Wanmeng Li helped with the bioinformatic data analysis. Lu Li, Yiqin Wang, and Xiaoting Chen conducted the AAV infusion experiments. Lin Bai and Ping Liao helped in the tissue-clearing technique. Yaguang Zhang provided technical assistance in ChIP experiments. Feng Li and Fengzhi Wu provided suggestions for study design related to natural products. Chenxia Han and Dan Du wrote the manuscript.

Conflicts of interest

The authors declare no competing interests.

Appendix A. Supplementary information

Supporting information to this article can be found online at <https://doi.org/10.1016/j.apsb.2025.03.037>.

References

- Mederos MA, Reber HA, Girgis MD. Acute pancreatitis: a review. *JAMA* 2021;**325**:382–90.
- Jaan A, Sarfraz Z, Farooq U, Malik S, Ur Rahman A, Okolo 3rd P. Incidence, implications and predictors of abdominal compartment syndrome in acute pancreatitis: a nationwide analysis. *Pancreatol* 2024;**24**:370–7.
- Barreto SG, Habtezion A, Gukovskaya A, Lugea A, Jeon C, Yadav D, et al. Critical thresholds: key to unlocking the door to the prevention and specific treatments for acute pancreatitis. *Gut* 2021;**70**:194–203.
- Zi Z, Rao Y. Discoveries of GPR39 as an evolutionarily conserved receptor for bile acids and of its involvement in biliary acute pancreatitis. *Sci Adv* 2024;**10**:eadj0146.
- Steinhoff MS, von Mentzer B, Geppetti P, Pothoulakis C, Bunnett NW. Tachykinins and their receptors: contributions to physiological control and the mechanisms of disease. *Physiol Rev* 2014;**94**:265–301.
- Padmanaban V, Keller I, Seltzer ES, Ostendorf BN, Kerner Z, Tavazoe SF. Neuronal substance P drives metastasis through an extracellular RNA–TLR7 axis. *Nature* 2024;**633**:207–15.
- Ramnath RD, Bhatia M. Substance P treatment stimulates chemokine synthesis in pancreatic acinar cells via the activation of NF- κ B. *Am J Physiol Gastrointest Liver Physiol* 2006;**291**:G1113–9.
- Zhu Z, Bhatia M. Inflammation and organ injury the role of substance P and its receptors. *Int J Mol Sci* 2023;**24**:6140.
- Schmidlin F, Dery O, Bunnett NW, Grady EF. Heterologous regulation of trafficking and signaling of G protein-coupled receptors: β -arrestin-dependent interactions between neurokinin receptors. *Proc Natl Acad Sci U S A* 2002;**99**:3324–9.
- Janetzko J, Kise R, Barsi-Rhine B, Siepe DH, Heydenreich FM, Kawakami K, et al. Membrane phosphoinositides regulate GPCR– β -arrestin complex assembly and dynamics. *Cell* 2022;**185**:4560–73.e19.
- DeFea KA, Vaughn ZD, O'Bryan EM, Nishijima D, Dery O, Bunnett NW. The proliferative and antiapoptotic effects of substance P are facilitated by formation of a β -arrestin-dependent scaffolding complex. *Proc Natl Acad Sci U S A* 2000;**97**:11086–91.
- Han C, Du D, Wen Y, Li J, Wang R, Jin T, et al. Chaiqin chengqi decoction ameliorates acute pancreatitis in mice via inhibition of neuron activation-mediated acinar cell SP/NK1R signaling pathways. *J Ethnopharmacol* 2021;**274**:114029.
- Kang J, Shi Y, Xiang B, Qu B, Su W, Zhu M, et al. A nuclear function of β -arrestin1 in GPCR signaling: regulation of histone acetylation and gene transcription. *Cell* 2005;**123**:833–47.
- Hoepfner CZ, Cheng N, Ye RD. Identification of a nuclear localization sequence in β -arrestin-1 and its functional implications. *J Biol Chem* 2012;**287**:8932–43.
- Petersen OH, Gerasimenko JV, Gerasimenko OV, Gryshchenko O, Peng S. The roles of calcium and ATP in the physiology and pathology of the exocrine pancreas. *Physiol Rev* 2021;**101**:1691–744.
- Biczko G, Vegh ET, Shalbueva N, Mareninova OA, Elperin J, Lotshaw E, et al. Mitochondrial dysfunction, through impaired autophagy, leads to endoplasmic reticulum stress, deregulated lipid metabolism, and pancreatitis in animal models. *Gastroenterology* 2018;**154**:689–703.
- Lee PJ, Papachristou GI. New insights into acute pancreatitis. *Nat Rev Gastroenterol Hepatol* 2019;**16**:479–96.
- Mullen NJ, Singh PK. Nucleotide metabolism: a pan-cancer metabolic dependency. *Nat Rev Cancer* 2023;**23**:275–94.
- Van den Berghe G, Bontemps F, Vincent MF, Van den Bergh F. The purine nucleotide cycle and its molecular defects. *Prog Neurobiol* 1992;**39**:547–61.
- Wen Y, Han C, Liu T, Wang R, Cai W, Yang J, et al. Chaiqin chengqi decoction alleviates severity of acute pancreatitis via inhibition of TLR4 and NLRP3 inflammasome: identification of bioactive ingredients via pharmacological sub-network analysis and experimental validation. *Phytomedicine* 2020;**79**:153328.
- Rong J, Han C, Huang Y, Wang Y, Qiu Q, Wang M, et al. Inhibition of xanthine oxidase alleviated pancreatic necrosis via HIF-1 α -regulated LDHA and NLRP3 signaling pathway in acute pancreatitis. *Acta Pharm Sin B* 2024;**14**:3591–604.
- Bai L, Wu Y, Dai W, Zhang J, Zheng L, Cheng J. A simple and effective vascular network labeling method for transparent tissues of mice. *J Biophotonics* 2023;**16**:e202300042.
- Xiao X, Guo P, Prasad K, Shiota C, Peirish L, Fischbach S, et al. Pancreatic cell tracing, lineage tagging and targeted genetic manipulations in multiple cell types using pancreatic ductal infusion of adeno-associated viral vectors and/or cell-tagging dyes. *Nat Protoc* 2014;**9**:2719–24.
- Murphy JA, Criddle DN, Sherwood M, Chvanov M, Mukherjee R, McLaughlin E, et al. Direct activation of cytosolic Ca²⁺ signaling and enzyme secretion by cholecystokinin in human pancreatic acinar cells. *Gastroenterology* 2008;**135**:632–41.
- Swain SM, Romac MJ, Shahid RA, Pandol SJ, Liedtke W, Vigna SR, et al. TRPV4 channel opening mediates pressure-induced pancreatitis initiated by Piezo1 activation. *J Clin Invest* 2020;**130**:2527–41.
- Navratilova E, Porreca F. Substance P and inflammatory pain: getting it wrong and right simultaneously. *Neuron* 2019;**101**:353–5.
- Yang JX, Shi N, Wang SS, Wang MJ, Huang Y, Wang YQ, et al. Multi-dimensional metabolomic profiling reveals dysregulated ornithine metabolism hallmarks associated with a severe acute pancreatitis phenotype. *Transl Res* 2024;**263**:28–44.
- Zarou MM, Rattigan KM, Sarnello D, Shokry E, Dawson A, Iannicello A, et al. Inhibition of mitochondrial folate metabolism drives differentiation through mTORC1 mediated purine sensing. *Nat Commun* 2024;**15**:1931.
- Martinez-Reyes I, Chandel NS. Mitochondrial TCA cycle metabolites control physiology and disease. *Nat Commun* 2020;**11**:102.
- Gooding JR, Jensen MV, Dai X, Wenner BR, Lu D, Arumugam R, et al. Adenylosuccinate is an insulin secretagogue derived from fluocose-induced purine metabolism. *Cell Rep* 2015;**13**:157–67.
- Recio R, Larena P, Pozo E, Calderon-Montano JM, Burgos-Moron E, Lopez-Lazaro M, et al. Carbohydrate-based NK1R antagonists with broad-spectrum anticancer activity. *J Med Chem* 2021;**64**:10350–70.
- Harris JA, Faust B, Gondin AB, Damgen MA, Suomivuori CM, Veldhuis NA, et al. Selective G protein signaling driven by substance P–neurokinin receptor dynamics. *Nat Chem Biol* 2022;**18**:109–15.
- Kleczkowska P, Nowicka K, Bujalska-Zadrozny M, Hermans E. Neurokinin-1 receptor-based bivalent drugs in pain management: the journey to nowhere?. *Pharmacol Ther* 2019;**196**:44–58.
- Liang G, Yang J, Liu T, Wang S, Wen Y, Han C, et al. A multi-strategy platform for quality control and Q-markers screen of Chaiqin chengqi decoction. *Phytomedicine* 2021;**85**:153525.
- Koh YH, Tamizhselvi R, Bhatia M. Extracellular signal-regulated kinase 1/2 and c-Jun NH2-terminal kinase, through nuclear factor- κ B and activator protein-1, contribute to caerulein-induced expression of substance P and neurokinin-1 receptors in pancreatic acinar cells. *J Pharmacol Exp Ther* 2010;**332**:940–8.
- Li B, Han X, Ye X, Ni J, Wu J, Dai J, et al. Substance P-regulated leukotriene B4 production promotes acute pancreatitis-associated lung injury through neutrophil reverse migration. *Int Immunopharmacol* 2018;**57**:147–56.
- Lindsay TH, Halvorson KG, Peters CM, Ghilardi JR, Kuskowski MA, Wong GY, et al. A quantitative analysis of the sensory and sympathetic innervation of the mouse pancreas. *Neuroscience* 2006;**137**:1417–26.
- Thorens B. Neuronal glucose sensing mechanisms and circuits in the control of insulin and glucagon secretion. *Physiol Rev* 2024;**104**:1461–86.

39. Liddle RA, Nathan JD. Neurogenic inflammation and pancreatitis. *Pancreatol* 2004;**4**:551–9; discussion 9–60.
40. Shahid RA, Vigna SR, Layne AC, Romac JM, Liddle RA. Acinar cell production of leukotriene B₄ contributes to development of neurogenic pancreatitis in mice. *Cell Mol Gastroenterol Hepatol* 2015;**1**:75–86.
41. Mitew S, Gobius I, Fenlon LR, McDougall SJ, Hawkes D, Xing YL, et al. Pharmacogenetic stimulation of neuronal activity increases myelination in an axon-specific manner. *Nat Commun* 2018;**9**:306.
42. Hua F, Wang HR, Bai YF, Sun JP, Wang WS, Xu Y, et al. Substance P promotes epidural fibrosis via induction of type 2 macrophages. *Neural Regen Res* 2023;**18**:2252–9.
43. Ramnath RD, Sun J, Bhatia M. Involvement of SRC family kinases in substance P-induced chemokine production in mouse pancreatic acinar cells and its significance in acute pancreatitis. *J Pharmacol Exp Ther* 2009;**329**:418–28.
44. Lau HY, Bhatia M. The effect of CP96,345 on the expression of tachykinins and neurokinin receptors in acute pancreatitis. *J Pathol* 2006;**208**:364–71.
45. Liu S, Liu H, Qin R, Shu Y, Liu Z, Zhang P, et al. The cellular senescence of leukemia-initiating cells from acute lymphoblastic leukemia is postponed by β -arrestin1 binding with P300-Sp1 to regulate hTERT transcription. *Cell Death Dis* 2017;**8**:e2756.
46. Qin R, Li K, Qi X, Zhou X, Wang L, Zhang P, et al. β -Arrestin1 promotes the progression of chronic myeloid leukaemia by regulating BCR/ABL H4 acetylation. *Br J Cancer* 2014;**111**:568–76.
47. Luo XM, Lam SM, Dong Y, Ma XJ, Yan C, Zhang YJ, et al. The purine metabolite inosine monophosphate accelerates myelopoiesis and acute pancreatitis progression. *Commun Biol* 2022;**5**:1088.
48. Wang XD, Yu WL, Sun Y. Activation of AMPK restored impaired autophagy and inhibited inflammation reaction by up-regulating SIRT1 in acute pancreatitis. *Life Sci* 2021;**277**:119435.
49. Wang K, Zhao A, Tayier D, Tan K, Song W, Cheng Q, et al. Activation of AMPK ameliorates acute severe pancreatitis by suppressing pancreatic acinar cell necroptosis in obese mice models. *Cell Death Discov* 2023;**9**:363.
50. Chong YC, Toh TB, Chan Z, Lin QXX, Thng DKH, Hooi L, et al. Targeted inhibition of purine metabolism is effective in suppressing hepatocellular carcinoma progression. *Hepatol Commun* 2020;**4**:1362–81.
51. Singh SX, Yang R, Roso K, Hansen LJ, Du C, Chen LH, et al. Purine synthesis inhibitor L-alanosine impairs mitochondrial function and stemness of brain tumor initiating cells. *Biomedicines* 2022;**10**:751.
52. Tran DH, Kim D, Kesavan R, Brown H, Dey T, Soflaee MH, et al. *De novo* and salvage purine synthesis pathways across tissues and tumors. *Cell* 2024;**187**:3602–18.
53. Cader MZ, de Almeida Rodrigues RP, West JA, Sewell GW, Md-Ibrahim MN, Reikine S, et al. FAMIN is a multifunctional purine enzyme enabling the purine nucleotide cycle. *Cell* 2020;**180**:815.
54. Wu Z, Bezada D, Cai F, Harris RC, Ko B, Sondhi V, et al. Electron transport chain inhibition increases cellular dependence on purine transport and salvage. *Cell Metab* 2024;**36**:1504–1520.e9.
55. Robles L, Vaziri ND, Li S, Takasu C, Masuda Y, Vo K, et al. Dimethyl fumarate ameliorates acute pancreatitis in rodent. *Pancreas* 2015;**44**:441–7.
56. Snider RM, Constantine JW, Lowe 3rd JA, Longo KP, Lebel WS, Woody HA, et al. A potent nonpeptide antagonist of the substance P (NK1) receptor. *Science* 1991;**251**:435–7.
57. Zernig G, Dietrich H, Maggi CA, Saria A. The substance P (NK1) receptor antagonist (+/-)-CP-96,345 causes sedation and motor impairment in Swiss albino mice in the black-and-white box behavioral paradigm. *Neurosci Lett* 1992;**143**:169–72.
58. Huang Y, Wen Y, Wang R, Hu L, Yang JX, Yang JQ, et al. Temporal metabolic trajectory analyzed by LC–MS/MS based targeted metabolomics in acute pancreatitis pathogenesis and Chaiqin Chengqi decoction therapy. *Phytomedicine* 2022;**99**:153996.
59. Chen Z, Yang X, Guo J, Jin T, Lin Z, Zhu P, et al. AGI grade-guided chaiqin chengqi decoction treatment for predicted moderately severe and severe acute pancreatitis (CAP trial): study protocol of a randomised, double-blind, placebo-controlled, parallel-group, pragmatic clinical trial. *Trials* 2022;**23**:933.
60. Lin Y, Li Y, Zeng Y, Tian B, Qu X, Yuan Q, et al. Pharmacology, toxicity, bioavailability, and formulation of magnolol: an update. *Front Pharmacol* 2021;**12**:632767.
61. Shen H, Liu S, Ding P, Wang L, Ju J, Liang G. Enhancement of oral bioavailability of magnolol by encapsulation in mixed micelles containing pluronic F127 and L61. *J Pharm Pharmacol* 2018;**70**:498–506.
62. Sheng YL, Xu JH, Shi CH, Li W, Xu HY, Li N, et al. UPLC–MS/MS-ESI assay for simultaneous determination of magnolol and honokiol in rat plasma: application to pharmacokinetic study after administration emulsion of the isomer. *J Ethnopharmacol* 2014;**155**:1568–74.
63. Wang X, Gu H, Zhang H, Xian J, Li J, Fu C, et al. Oral core-shell nanoparticles embedded in hydrogel microspheres for the efficient site-specific delivery of magnolol and enhanced antitumor colitis therapy. *ACS Appl Mater Inter* 2021;**13**:33948–61.
64. Nuber S, Zabel U, Lorenz K, Nuber A, Milligan G, Tobin AB, et al. β -Arrestin biosensors reveal a rapid, receptor-dependent activation/deactivation cycle. *Nature* 2016;**531**:661–4.