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Original Research

Notch1 Inhibition Exacerbates APAP-Induced Liver Injury via β-Catenin and Macrophage Polarization

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Abstract

Background: Notch1 signaling regulates innate immune-mediated inflammation in acute liver injury (ALI). However, the precise mechanism by which Notch1 governs macrophage polarization during ALI remains poorly understood. **Methods**: Wild-type (WT) mice received DAPT (10 mg/kg) prior to acetaminophen (APAP)-induced ALI. In parallel, bone marrow-derived macrophages (BMMs) were pretreated with either the β-catenin inhibitor XAV939 or the activator SKL2001, exposed to DAPT, and then challenged with lipopolysaccharide (LPS). Liver injury and inflammation were evaluated by hematoxylin and eosin (H&E) staining, terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay, immunohistochemistry, immunofluorescence, quantitative real-time PCR (RT-PCR), and western blotting. **Results**: Unexpectedly, DAPT treatment exacerbated APAP-induced liver injury (AILI), resulting in more severe hepatocellular damage and inflammation than in controls. DAPT-treated macrophages exhibited enhanced pro-inflammatory cytokines expression and a shift toward an M1-like phenotype. Mechanistically, the β-catenin/glycogen synthase kinase 3 beta (GSK3β) signaling pathway emerged as a pivotal regulator of macrophage polarization. **Conclusions**: Notch1 inhibition unexpectedly worsens AILI by amplifying macrophage-driven pro-inflammatory responses via β-catenin signaling. These findings highlight the Notch1–β-catenin axis as a key regulator of hepatic macrophage function and a potential therapeutic target for sterile liver inflammation.

Keywords: APAP; β-catenin; DAPT; macrophage; Notch1; liver inflammation

1. Introduction

Drug-induced liver injury (DILI) represents the predominant cause of acute liver failure (ALF) in Western countries [1], with nearly half of cases (approximately 46%) arise from acetaminophen (APAP) overdose in the United States [2,3]. The hepatotoxicity effects of APAP arise from its metabolite NAPQI (N-acetyl-p-benzoquinone imine), which exhausts glutathione, impairs mitochondrial function, and triggers DNA injury [4]. The resulting hepatocyte damage provokes a robust sterile inflammatory response, largely orchestrated by hepatic macrophages and neutrophils through recognizing damage-associated molecular patterns (DAMPs), which further aggravate liver injury [4].

The evolutionarily conserved Notch signaling pathway is elemental for tissue homeostasis, stem cell maintenance, and regulation of crucial cellular processes such as proliferation, survival, apoptosis, and differentiation [5,6]. Dysregulation of Notch signaling has been implicated in diverse hepatic pathologies, including fibrosis and acute liver injury, where it influences hepatocyte function and immune cell behavior [7–9]. Notch1, in particular, has been shown to regulate the innate immune-mediated liver injury induced by APAP or hepatic ischemia/reperfusion (I/R) in our previous [4,10,11]. Notably, Notch1 signaling influences macrophage polarization in a context-dependent manner [12]. Pharmacologic or genetic inhibition of Notch1 can attenuate M1-driven fibroblast activation and fibroblast-induced M1 polarization [13], while also affecting M2 differentiation [14]. Nevertheless, the precise mechanisms by which Notch1 governs macrophage polarization during APAP-induced liver injury remain unclear.

Crosstalk between Notch1 and β-catenin signaling has emerged as a potential mechanism regulating macrophage function [15]. Wnt/β-catenin signaling is essential for maintaining tissue homeostasis [16], with β-catenin and its transcriptional coactivators controlling genes involved in proliferation, survival, differentiation, and migration [17]. In macrophages, Notch1 can modulate transforming growth factor beta-activated kinase 1 (TAK1)-mediated innate immunity through β-catenin signaling [15]. Furthermore, in hepatocellular carcinoma, Nicastrin (NCSTN) promotes tumor progression and metastasis via Notch1/Protein kinase B (AKT) signaling, which upregulates Zeb1 through β-catenin [18]. Although Notch–β-catenin interactions have been implicated in I/R stress and hepatocellular carcinoma

Table 1. Primer sequences for the amplification.

Target genes	Forward primers	Reverse primers
mβ-Actin	5′-GTGACGTTGACATCCGTAAAGA-3′	5′-GCCGGACTCATCGTACTCC-3′
mTnf-α	5′-ACGGCATGGATCTCAAAGAC-3′	5′-AGATAGCAAATCGGCTGACG-3′
mIL-6	5′-GCTACCAAACTGGATATAATCAGGA-3′	5′-CCAGGTAGCTATGGTACTCCAGAA-3′
mMcp-1	5′-TGCTTCTGGGCCTGCTGTTC-3′	5′-ACCTGCTGCTGGTGATCCTCT-3′
mArg-1	5′-GACACCCATCCTATCACCGC-3′	5′-GCGGCTGTGCATCATACAAC-3′
mIL-10	5′-GCCAGTACAGCCGGGAAGAC-3′	5′-GCCGATGATCTCTCTCAAGTGAT-3′

(HCC) progression, their cooperative role in APAP-induced liver inflammation remains not clear.

In this study, we demonstrate that pharmacological inhibition of Notch1 unexpectedly exacerbates APAP-induced liver injury (AILI) by driving macrophages toward a pro-inflammatory M1 phenotype via β-catenin/GSK3β signaling. Collectively, these findings uncover a previously unrecognized Notch1-β-catenin regulatory axis in liver inflammation and identify β-catenin as a potential therapeutic target in DILI.

2. Materials and Methods

2.1 Animal Experiments

Wild-type (WT) mice (C57BL/6, male, 8 weeks) were obtained from the Animal Core Facility of Nanjing Medical University (Nanjing, China). Mice were kept in SPF conditions with a 12-h light/dark cycle and ad libitum access to food and water. All procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of Nanjing Medical University (IACUC-2206039) and conducted in accordance with NIH *Guide for the Care and Use of Laboratory Animals*.

ALI was conducted by intraperitoneal (i.p.) injection of APAP (400 mg/kg, 1003009, Sigma-Aldrich, St. Louis, MO, USA) [4]. In the treatment group, mice were administered the DAPT (10 mg/kg, D5942, Sigma-Aldrich, St. Louis, MO, USA), the γ-secretase inhibitor, via tail vein injection 30 minutes prior to APAP administration. Animals were euthanized at predetermined time points by cervical dislocation following anesthesia with ketamine (90 mg/kg) and xylazine (15 mg/kg). Blood and liver tissues were collected for subsequent biochemical, histological, and molecular analyses.

2.2 Serum Biochemistry

Blood samples were centrifuged to obtain serum. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were measured using a commercial assay kit (IDEXX Laboratories, Westbrook, ME, USA) from serum, according to the manufacturer's instructions.

2.3 Histology and Immunohistochemistry

Liver samples were fixed in 10% neutral buffered formalin for 24 h, embedded in paraffin, and then sectioned at 5 µm. The slides were stained with Hematoxylin and eosin (H&E) for histological evaluation. Macrophage infiltrations were detected by immunohistochemistry using a CD11b monoclonal antibody (1:100, MCA711, Bio-RAD, Hercules, CA, USA) and visualize with the ABC Kit (Vector, PK-7200). CD11b-positive cells were quantified in 10 randomly selected high-power fields (HPFs) by a blinded observer.

2.4 Immunofluorescence

Frozen liver sections (5 µm) or cultured cells were fixed in formalin, blocked with 5% BSA, and incubated overnight at 4 °C with primary antibodies, including rabbit anti-iNOS (1:100, 13120, Cell Signaling Technology, Danvers, MA, USA), rabbit anti-CD206 (1:100, 24595, Cell Signaling Technology, Danvers, MA, USA), and mouse anti-CD163 (1:100, sc-58965, Santa Cruz, Dallas, TX, USA). Samples were then incubated with Cy™5- or AlexFluor488-conjugated secondary antibodies (1:250, Jackson ImmunoResearch, West Grove, PA, USA) for 1 h and nuclei were counterstaining with DAPI (sc-24941, Santa Cruz, Dallas, TX, USA). Images were captured using a LEICA DMI3000B microscope (Leica Microsystems, Wetzlar, Germany) and analyzed with ImageJ, version 2.9/1.53t (NIH, Bethesda, MD, USA).

2.5 TUNEL Detection

Hepatic cell death was detected using the Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay with the Klenow-FragEL DNA Detection Kit (QIA33, EMD Chemicals or MilliporeSigma, St. Louis, MO, USA), following manufacturer's instructions [15]. Positive cells were quantified in 10 randomly selected HPFs.

2.6 Quantitative Real-time PCR (RT-qPCR)

Total RNA (2.5 µg) was reverse using the SuperScript™ III Reverse Transcriptase System (18080051, Invitrogen, Waltham, MA, USA) [4]. RT-qPCR was performed using the Platinum SYBR Green qPCR Kit (11736059, Invitrogen) under the following conditions: 50 °C for 2 minutes, 95 °C for 5 minutes, followed by 40 cycles of 95 °C for 15 seconds and 60 °C for 30 seconds. Primer sequences are provided in Table 1.

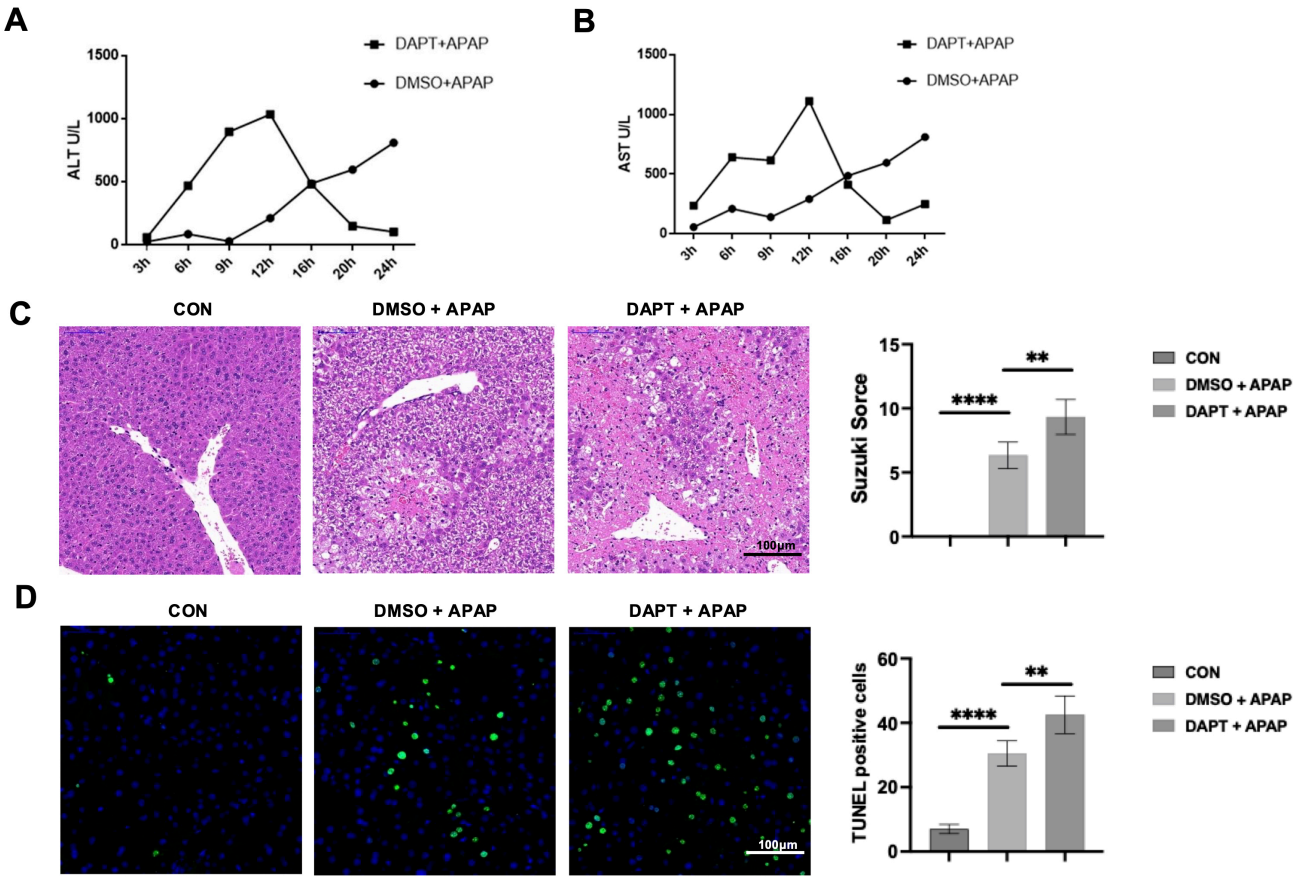

Fig. 1. Inhibition of Notch1 signaling exacerbates acetaminophen (APAP)-induced liver injury. Mice received a tail vein injection of the Dimethyl sulfoxide (DMSO) or γ-secretase inhibitor DAPT 30 minutes prior to APAP administration. (A,B) Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) (IU/L) levels at 3 hours, 6 hours, 9 hours, 12 hours, 16 hours, 20 hours and 24 hours after APAP administration, respectively. (n = 6/group). (C) Representative Hematoxylin and eosin (H&E) staining at 12 hours and Suzuki's score. (n = 6/group). Scale bar, 100 µm. (D) Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining and quantification of hepatocytes cell death (green, n = 6/group). Scale bar, 100 µm. $^{**}p < 0.01$, $^{****}p < 0.0001$.

2.7 Western Blotting

Proteins (30 µg/sample) from liver tissue or cultured cells were separated using 12% SDS-PAGE and then transferred to a nitrocellulose membrane (Bio-Rad) and probed with antibodies against monoclonal anti-rabbit STAT1 (1:1000, 14994, Cell Signaling Technology, Danvers, MA, USA), phos (p)-STAT1 (1:1000, 9167, Cell Signaling Technology, Danvers, MA, USA), STAT6 (1:1000, 5397, Cell Signaling Technology, Danvers, MA, USA), phos-STAT6 (1:1000, 56554, Cell Signaling Technology, Danvers, MA, USA), NICD (1:1000, 3608, Cell Signaling Technology, Danvers, MA, USA), β-catenin (1:1000, 8480, Cell Signaling Technology, Danvers, MA, USA), GSK3β (1:1000, 12456, Cell Signaling Technology, Danvers, MA, USA), phos-GSK3β (1:1000, 5558, Cell Signaling Technology, Danvers, MA, USA), and β-actin Abs (1:1000, 12262, Cell Signaling Technology, Danvers, MA, USA). The analysis of images was conducted using ImageJ (NIH) and β-actin was used as loading control.

2.8 Bone Marrow-Derived Macrophage (BMM) Isolation and In Vitro Treatment

BMMs were extracted from the femurs and tibias of WT mice and cultured in Dulbecco's modified Eagle's medium (DMEM, GIBCO, Waltham, MA, USA, 11995-065) containing 10% FBS (GIBCO, FBS-500) and 15% L929-conditioned medium [4]. In certain experiments, BMMs were pre-treated with a β-catenin inhibitor XAV939 (HY-15147, MCE, Monmouth Junction, NJ, USA) or activator SKL2001 (HY-101085, MCE), followed by incubation with DAPT (10 µM). After 48 hours, the cells were stimulated with lipopolysaccharide (LPS) (100 ng/mL) (Sigma-Aldrich, *Escherichia coli O111: B4*) for six hours. All primary cells were validated for their identity by surface marker analysis using flow cytometry and tested negative for mycoplasma.

2.9 Statistical Analysis

Data are expressed as mean ± SD. For comparisons between two-groups, Student's *t* tests were applied, while

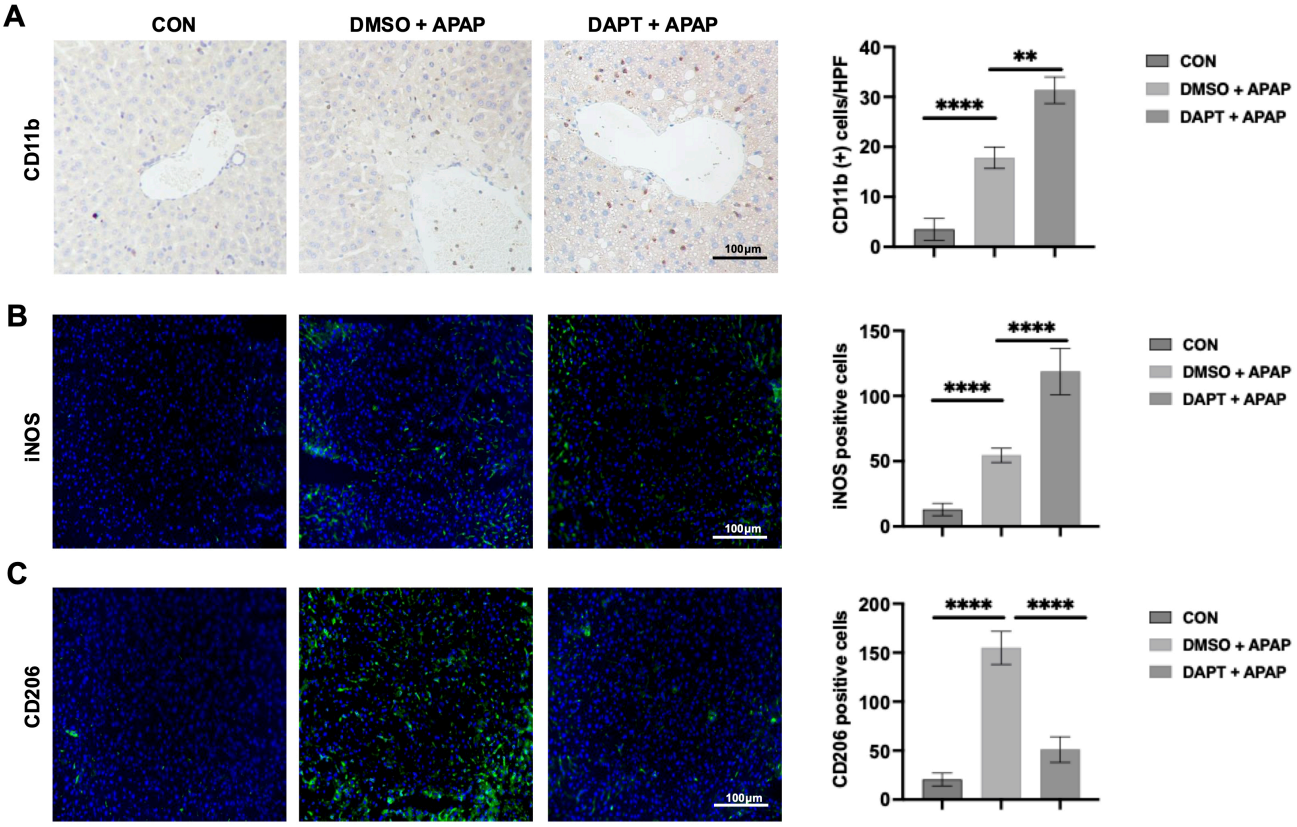

Fig. 2. Inhibition of Notch1 signaling increases hepatic macrophage infiltration and polarization. (A) Immunohistochemical staining and 1uantification of $CD11b^+$ macrophages in liver tissue 12 hours after APAP-challenged. (n = 6/group). Scale bar, 100 μm. (B) Immunofluorescence staining and quantification of inducible nitric oxide synthase ($iNOS^+$) macrophages (n = 6/group). Scale bar, 100 μm. (C) Immunofluorescence staining and quantification of $CD206^+$ macrophages (n = 6/group). Scale bar, 100 μm. $^{**}p < 0.01$, $^{****}p < 0.0001$.

differences among more than two groups were evaluated by one-way analysis of variance (ANOVA) followed by Tukey's post hoc testing. A two-sided p-value < 0.05 was considered statistically significant.

3. Results

3.1 Inhibition of Notch1 Signaling Exacerbates APAP-induced Liver Injury

We firstly employed a mouse model of AILI to investigate Notch1-mediated hepatic injury. Mice pretreated with the γ-secretase inhibitor DAPT displayed significantly higher serum ALT and AST levels than Dimethyl sulfoxide (DMSO)-treated controls, reaching their maximum values at 12 h post-APAP (Fig. 1A,B). Histopathological analysis of H&E-stained paraffin liver sections at this time point demonstrated more severe cell death, congestion, and vacuolar degeneration in DAPT-treated mice, as quantified by Suzuki's score (Fig. 1C). Consistently, TUNEL staining confirmed increased hepatic cell death in the DAPT-treated mice (Fig. 1D). Collectively, these data indicate that Notch1 inhibition with DAPT aggravated hepatocellular injury induce by APAP.

3.2 Inhibition of Notch1 Signaling Increases Hepatic Macrophage Infiltration and Polarization

We next evaluated the impact of Notch1 inhibition on macrophage accumulation during AILI. Immunohistochemical staining for CD11b showed marked macrophage infiltration in APAP-injured livers, with DAPT treatment further increasing $CD11b^+$ macrophage accumulation, especially in the portal regions (Fig. 2A). Immunofluorescence further analysis revealed a higher abundance of $iNOS^+$ macrophages (M1-like) (Fig. 2B) accompanied by a reduction of $CD206^+$ macrophages (M2-like) (Fig. 2C) in the DAPT-treated group relative to controls. This suggests that Notch1 signaling normally restrains macrophage recruitment while favoring M2 polarization during AILI.

3.3 Inhibition of Notch1 Signaling Promotes a Pro-inflammatory Cytokine Profile

We then used BMMs were challenged with LPS with or without DAPT to assess whether blockade of Notch1 influences macrophage activation. Compared with LPS controls, DAPT-treated BMMs exhibited significantly higher mRNA levels of the pro-inflammatory mediators *TNF-α*, *IL-6*, and *MCP-1* (Fig. 3A–C), accompanied by di-

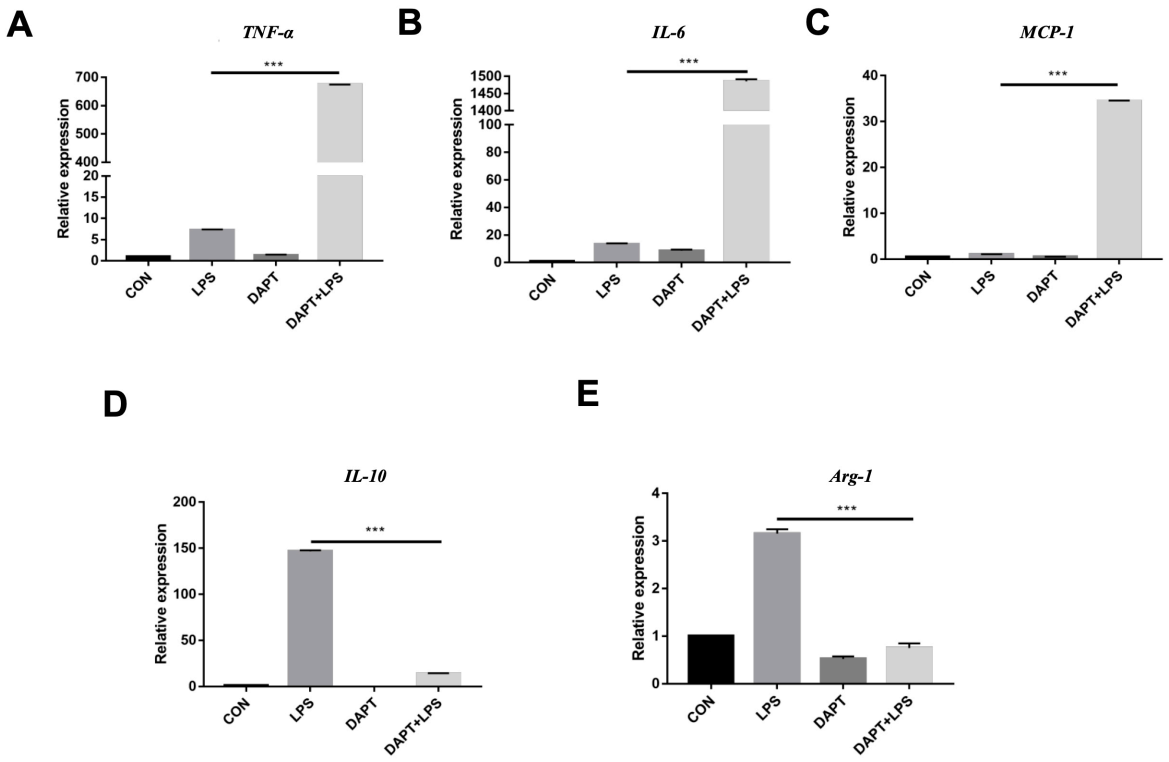

Fig. 3. Inhibition of Notch1 signaling promotes a pro-inflammatory cytokine profile. (A–C) Quantitative real-time PCR (RT-qPCR) analysis of *TNF-α*, *IL-6*, and *MCP-1* expression in bone marrow-derived macrophages (BMMs). (D,E) RT-qPCR analysis of *IL-10* and *Arg-1* expression in BMMs. Data presented mean ± SD (n = 6/group). ***$p < 0.001$.

minished expression of the anti-inflammatory cytokines *IL-10* and *Arg-1* (Fig. 3D,E). In addition, we also assessed cytokine genes expression in BMMs exposed to DAPT following a 6 h challenge with APAP (10 mM) [19]. Relative to APAP-challenged controls, DAPT-treated BMMs displayed significantly higher expression of pro-inflammatory mediators *TNF-α*, *IL-6*, and *MCP-1* (**Supplementary Fig. 1A–C**). Conversely, the anti-inflammatory cytokines *IL-10* and *Arg-1* levels was decreased (**Supplementary Fig. 1D,E**). Collectively, these findings indicate that macrophage Notch1 inhibition promotes a pro-inflammatory profile in stress environment.

3.4 Notch1 Blockade Promotes M1 Polarization and Suppresses M2 Polarization

To further explore whether BMMs differentiate into the M1 pro-inflammatory phenotype, we then measured the expression of specific biomarkers associated with M1 and M2 macrophage polarization. Western blot analysis revealed that DAPT treatment increased p-STAT1 (M1-associated) while decreasing p-STAT6 (M2-associated) [20] levels in BMMs after LPS stimulation (Fig. 4A). Immunofluorescence revealed a higher proportion of iNOS$^+$ macrophages (Fig. 4B) and a decrease in CD206$^+$ macrophages (Fig. 4C) in DAPT-treated group. Together, these results suggest that Notch1 promotes M2 polarization while restraining M1 activation.

3.5 Notch1 Inhibition Suppresses β-catenin/GSK3β Signaling

To investigate the underlying mechanism, we examined β-catenin signaling in BMMs pretreated with DAPT. Compared with controls, Notch1 blockade led to reduced levels of β-catenin and p-GSK3β (Fig. 5), suggesting that Notch1 positively regulates the β-catenin pathway during macrophage polarization.

3.6 β-catenin Mediates Notch1-dependent Macrophage Polarization

To confirm whether β-catenin activity is essential for Notch1-mediated polarization, BMMs were pretreated with the β-catenin inhibitor XAV939 or a β-catenin agonist, SKL2001. Treatment with XAV939 resulted in reduced β-catenin and p-GSK3β expression, increased p-STAT1, and a decrease in p-STAT6, consistent with M1 polarization. In contrast, treatment with SKL2001 leads to increased β-catenin and p-GSK3β, a decrease in p-STAT1, and an increase in p-STAT6, favoring M2 polarization (Fig. 6A,B). Immunofluorescence analysis confirmed that XAV939 increased iNOS$^+$ macrophages and reduced CD163$^+$ macrophages. Conversely, SKL2001 showed the opposite effect (Fig. 6C,D). These results identify the Notch1-β-catenin axis as a central regulator of macrophage polarization in AILI.

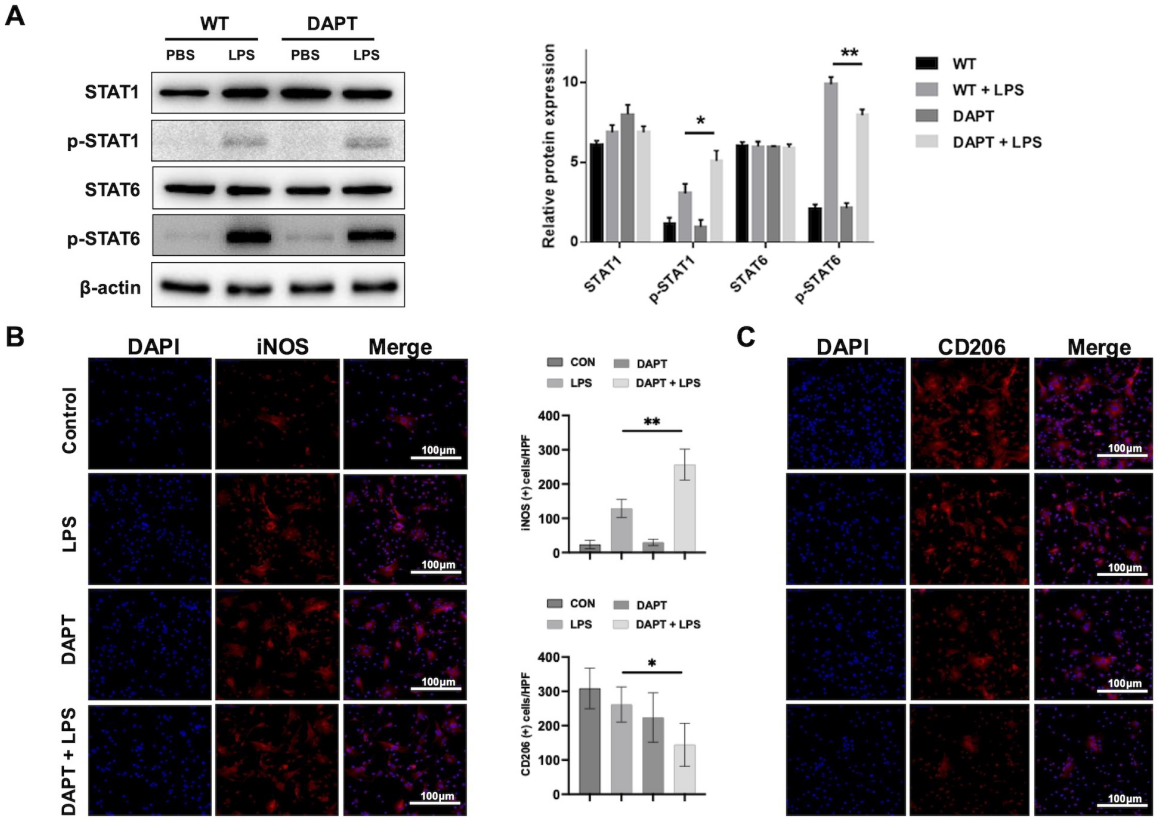

Fig. 4. Notch1 blockade promotes M1 polarization and suppresses M2 polarization. (A) Western blot and densitometric analysis of signal transducer and activator of transcription 1 (STAT1)/phos-STAT1, and STAT6/phos-STAT6 in lipopolysaccharide (LPS)-challenged BMMs with or without DAPT precondition. (B) Immunofluorescence staining and quantification of iNOS$^+$ macrophages (n = 6/group). Scale bar, 100 μm. (C) Immunofluorescence staining and quantification of CD206$^+$ macrophages (n = 6/group). Scale bar, 100 μm. *$p < 0.05$, **$p < 0.01$.

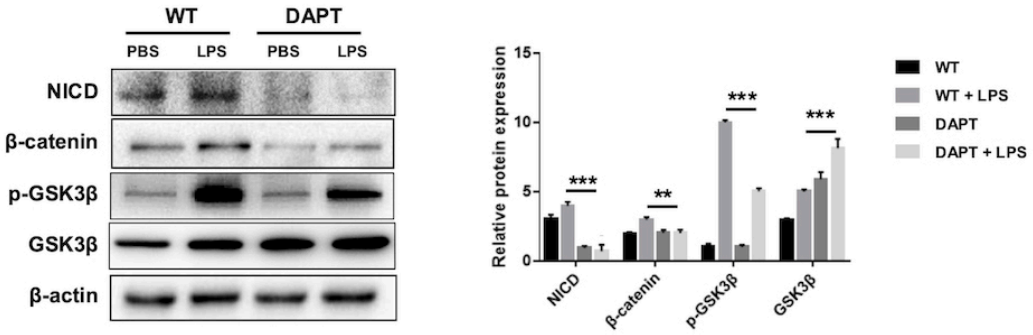

Fig. 5. Notch1 inhibition suppresses β-catenin/glycogen synthase kinase-3beta (GSK3β) signaling. Western blot and densitometric analysis of Notch intracellular domain (NICD), β-catenin, and GSK3β/phos-GSK3β in BMMs (n = 6/group). **$p < 0.01$, ***$p < 0.001$.

4. Discussion

The innate immune response is a critical driver of AILI [2,4]. Accumulating evidence demonstrates that toxic metabolites of APAP initially induce necrosis and apoptosis of hepatocytes [21,22], which subsequently activate innate immunity and stimulate macrophages to release pro-inflammatory cytokines. The upregulation of these inflammatory factors further exacerbates liver damage [23]. Given their pivotal role in mediating inflammatory injury, macrophages have been recognized as potential therapeutic targets in DILI. Herein, we identify the Notch1-β-catenin axis as a pivotal regulator of innate immunity responses and macrophage polarization in the context of APAP challenge.

Notch signaling is well-established mediator in liver pathology. It is activated in liver fibrosis and is found to be abnormally elevated in patients with fibrotic disease. In-

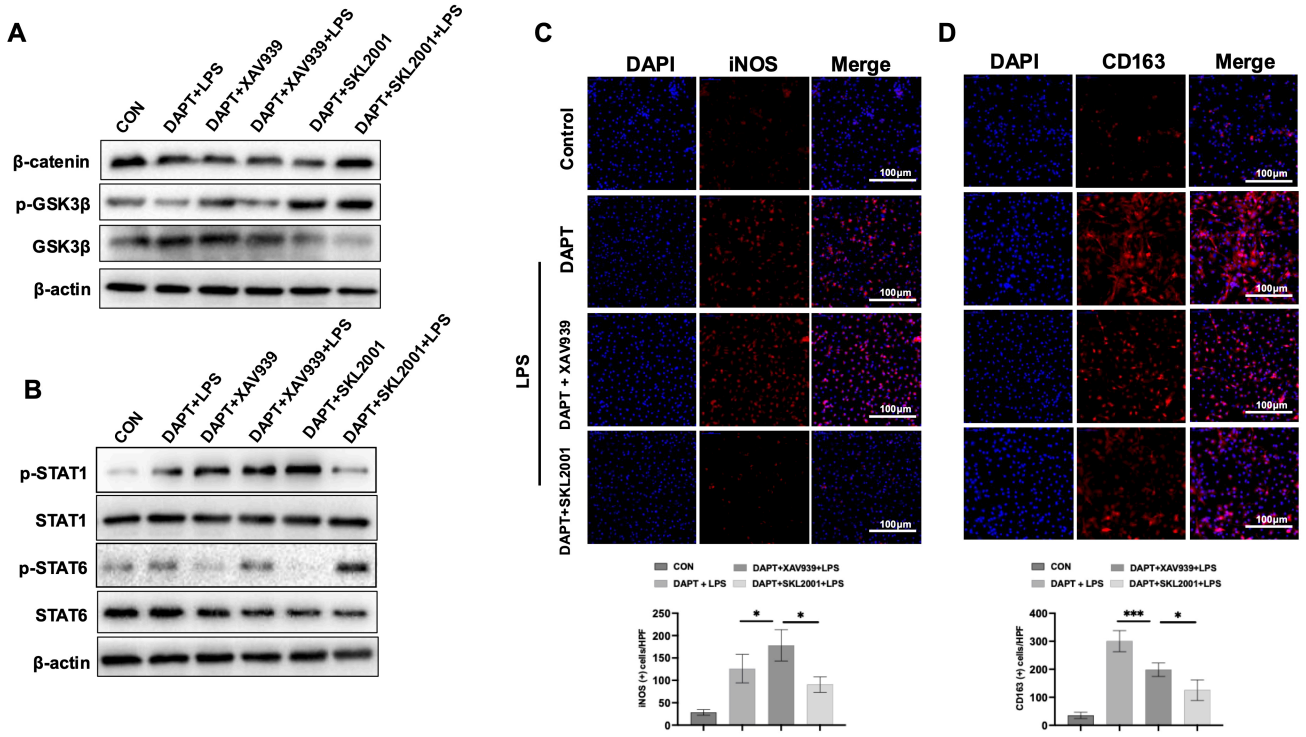

Fig. 6. β-catenin signaling mediates Notch1-dependent macrophage polarization. (A) Western blot and densitometric analysis of β-catenin, and GSK3β/phos-GSK3β in BMMs (n = 6/group). (B) Western blot and densitometric analysis of STAT1/phos-STAT1, and STAT6/phos-STAT6 in BMMs (n = 6/group). (C) Immunofluorescence staining and quantification of iNOS$^+$ macrophages (n = 6/group). Scale bar, 100 μm. (D) Immunofluorescence staining and quantification of CD163$^+$ macrophages (n = 6/group). Scale bar, 100 μm. *$p < 0.05$, ***$p < 0.001$.

hibiting this pathway can suppress the progression of fibrosis [24,25]. Our previous study demonstrated that Notch1 ablation exacerbated liver injury, a phenomenon linked to the activation of either NLR family pyrin domain containing 3 (NLRP3) [10] or stimulator of interferon genes (STING) signaling [4] in macrophage. Collectively, these findings support a role for Notch1 signaling in controlling macrophage activation during acute hepatic inflammation.

Macrophages exhibit remarkable plasticity, polarizing toward pro-inflammatory or anti-inflammatory phenotypes, contributing to diverse immune responses [26]. For example, Notch1 deletion has been reported to attenuate both M1-driven fibroblast activation and fibroblast-induced M1 polarization, while also limiting macrophage activation toward the M2 phenotype [13]. Conversely, some studies report that Notch1 inhibition can promote M2 polarization in injured livers [27]. In alcoholic liver disease, myeloid Notch1 knockout prevents macrophage infiltration and leads to M1 phenotype [28]. This suggests that Notch1-mediated macrophage function is highly dependent on the specific stress or disease environment. Nonetheless, whether pharmacologic Notch1 inhibition affects macrophage polarization in AILI has not been thoroughly investigated.

Our results demonstrated a marked increase hepatic macrophages following APAP treatment. Furthermore, with numbers further elevated in the DAPT-treated group. Previous studies have demonstrated that the resident macrophage Kupffer cells (KCs), which originate from specific progenitor cells in the yolk sac during embryonic development. These KCs maintain their population through self-renewal and are not dependent on monocyte-derived macrophages for replenishment [29]. During acute liver injury, activated KCs release inflammatory cytokines that recruit peripheral monocytes to the liver [30]. In our study, CD11b staining confirmed a marked increase in macrophages in injured liver. In the fulminant hepatitis mouse model, there was a significant depletion of KCs in the later stages, but an initial pro-inflammatory M1 polarization of macrophages was evident early on. Following the depletion of KCs, a large influx of peripheral monocytes into the liver was observed. These monocytes initially adopted a pro-inflammatory M1 phenotype but transitioned to an anti-inflammatory M2 phenotype in the later stages, promoting tissue repair and suppressing inflammation [31]. In the APAP mouse model, both macrophages are mainly distributed in the portal area [32]. In this study, Western blot and immunofluorescence analysis of BMMs treated with DAPT revealed increased M1 markers and de-

creased M2 markers, indicating that DAPT promotes M1 polarization. Correspondingly, DAPT-treated macrophages express higher levels of pro-inflammatory genes, such as *TNF-α*, while inhibit anti-inflammatory mediators' expression, including *IL-10*. Nevertheless, the precise mechanism by which Notch1 signaling regulates macrophage polarization remains unclear.

The β-catenin plays well-established roles in hepatic biology, including hepatocyte proliferation, regeneration, and tumorigenesis [33]. In APAP injury, β-catenin has been shown to facilitate regenerative responses [34] and to influence both monocyte infiltration and macrophage polarization during acute hepatic injury [35]. For example, scavenger receptor 1 (MSR1) in macrophage could promote M2 polarization through the activation of β-catenin [36]. In our study, DAPT reduced β-catenin and p-GSK3β levels in macrophages. Pharmacologic manipulation confirmed its role, β-catenin inhibition using XAV939 promoted M1 polarization, whereas activation with SKL2001 shifted polarization toward M2 phenotype. Immunofluorescence for iNOS and CD163 corroborated these shifts. These results establish β-catenin/GSK3β as a downstream effector of Notch1 in controlling macrophage polarization.

Undeniably, this study is subject to certain limitations. First, our findings are derived from murine models of APAP-induced liver injury, which may not entirely reflect the complexity of human DILI. Second, while our study focused on the Notch1-β-catenin axis in hepatic macrophages, other immune cell subsets and signaling pathways (such as NF-κB, JAK/STAT, and NLRP3 pathways) may also contribute to the observed phenotypes but were not explored in depth. Third, the temporal resolution of macrophage polarization was limited, and dynamic transition from acute injury to resolution remains unexplored. Future studies that integrate single-cell transcriptomics, lineage-tracing models, myeloid-specific Notch1 knockout mice models and human liver tissue analyses will be critical to validate these findings, define context-specific regulatory mechanisms, and evaluate the therapeutic potential of targeting Notch1-β-catenin axis in clinical managements.

5. Conclusion

In this study, we demonstrate that pharmacological inhibition of Notch1 in AILI promotes macrophage infiltration, drives toward a M1 phenotype, and exacerbates liver injury. This effect occurs, at least in part, due to suppression of β-catenin/GSK3β signaling. Our findings highlight Notch1 as a potential therapeutic target in drug-induced liver injury and suggest that selective modulation of macrophage function could guide future clinical strategies.

Availability of Data and Materials

The data supporting the findings of this study are available within the article and Supplementary Material. All data is available from the corresponding authors upon reasonable request.

Author Contributions

TY, PS and JL conceived and designed the study. PS, JJD, YYS and XW performed the animal experiments. PS, JJD, JYZ, and JLZ performed the experiments *in vitro*. TY and LFJ analyzed the data and drafted the manuscript. JL and LFJ modified the manuscript. All authors read and approved the final version of the manuscript. All authors contributed to editorial changes in the manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

The animal study was reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of Nanjing Medical University (IACUC-2206039) and conducted in accordance with NIH *Guide for the Care and Use of Laboratory Animals*.

Acknowledgment

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.31083/FBL43853.

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