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Original Article

Different effects of TCBPA exposure on liver cancer cells and liver cells: two sides of the coin

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Abstract: Tetrachlorobisphenol A (TCBPA), widely used as a substitute for tetrabromobisphenol A (TBBPA), has been detected in various environmental media. Therefore, a detailed evaluation of the toxicological properties of TCBPA is necessary. In this study, we used hepatoma and normal liver cell models *in vitro* to investigate the effects of TCBPA. Our findings indicate that TCBPA promotes the proliferation of liver cancer cells, as evidenced by MTT and EdU assays, and enhances the expression levels of molecules related to hepatoma proliferation. Further investigation into the molecular mechanism revealed that TCBPA-induced hepatoma proliferation is regulated by an NLRP3-mediated inflammatory process. Additionally, TCBPA was found to promote the epithelial-mesenchymal transition (EMT) process in liver cancer cells. Conversely, TCBPA inhibited the proliferation of normal liver cells. Mechanistic studies showed that TCBPA induced cell pyroptosis in normal liver cells by evaluating a series of related markers, including NLRP3, IL-1β, ASC, GASDMD, and Caspase 1. *In vivo* models further showed that TCBPA causes liver tissue damage. In summary, this study demonstrates that TCBPA has a dual effect: promoting the occurrence and development of liver tumor cells in vitro, while inhibiting the proliferation of normal liver cells, like two sides of a coin. These opposite cellular outcomes are regulated by NLRP3-mediated inflammatory processes, providing valuable insights for evaluating the potential health impacts of TCBPA.

Keywords: TCBPA, hepatocellular carcinoma cells, liver cell, proliferation, pyroptosis

Introduction

The production and application of brominated flame retardants (BFRs) have spanned several decades [1]. Typical BFRs include polybrominated diphenylethers (PBDEs), hexabromocyclododecanes (HBCD), and tetrabromobisphenol A (TBBPA) [2]. BFRs are extensively used as chemical additives in consumer products, such as electronics and textiles, to reduce flammability [3]. Among these, TBBPA is one of the most prevalently used brominated flame retardants. However, due to its biological toxicity, its use has been banned in many countries [4]. As such, Tetrachlorobisphenol A (TCBPA) and tetrabromobisphenol S (TBBPS) have been widely used as alternatives to TBBPA. TCBPA, found in building materials, coatings, synthetic plastics, and electronic devices, is inevitably released into the environment, where it persists and accumulates in organisms due to its hydrophobic nature, impacting both these organisms and human health overall [5]. Studies have shown that TCBPA has been detected in various environmental media due to its lipophilicity and environmental persistence. TCBPA has been found in human blood, breast milk, urine samples, and even in pregnant women and their embryos, raising concerns over potential health threats [6].

Humans are exposed to TCBPA through diet, ingestion, skin contact with dust, and air inhalation, with the pollutant being detected in human samples [7]. For example, TCBPA was found in 18.6% of serum samples from 150 female volunteers [7]. Various studies have evaluated the toxicological effects of TCBPA using *in vitro* and

in vivo models; these studies have indicated its potential for endocrine disruption, cytotoxicity, liver toxicity, and neurotoxicity [8]. For example, using zebrafish as an aquatic model organism, research has found that TCBPA has toxic effects on the cardiovascular and nervous systems of zebrafish [9]. Further, Wang et al. reported that TCBPA caused immunosuppression in a mouse model [7].

The liver is the most important digestive organ in humans and animals, and functions to synthesize and secrete bile, store glycogen, and regulate important biological molecules such as protein, fat, and carbohydrates [10]. Various environmental factors can induce liver carcinogenesis. Hepatocellular carcinoma is the sixth most common primary cancer in humans, and its mortality rate ranks second among all cancers [11, 12]. Hepatocellular carcinoma is characterized by high recurrence, complexity, invasive metastasis, and delayed diagnosis, with a low five-year survival rate. There are many factors that contribute to hepatocellular carcinoma, including environmental pollution factors [13]. Studies have shown that pollutants and toxic substances in the environment have a significant impact on genesis and development of hepatocellular carcinoma [14]. Given the widespread environmental and human presence of TCBPA, it is necessary to assess the potential effects of TCBPA on the liver and liver cancer.

In this work, we examined the effect of TCBPA on liver cells and liver cancer cells, measuring changes in biochemical indicators after TCBPA exposure *in vitro* and *in vivo*. Here, we showed that TCBPA exposure can promote the malignant progression of liver cancer cells. In our liver cell model, TCBPA exposure led to liver cell injury. The observations in this study offers valuable information for evaluating the potential health impacts of TCBPA.

Materials and methods

Antibodies and reagents

Dulbecco's Modified Eagle Medium (DMEM) was sourced from Thermo Fisher Scientific (USA). The LDH Release Assay Kit (Catalog Number: C0017) and the TUNEL Staining Kit (BeyoClick™ EdU-555) were purchased from Beyotime (Shanghai, China). The following antibodies were purchased from Beyotime: NLRP3 Rabbit Monoclonal Antibody (Number: AF2155), Caspase-1 Rabbit Monoclonal Antibody (AF1681), ZO-1 Rabbit Polyclonal Antibody (AF8394), Claudin-1 Rabbit Monoclonal Antibody (AG4424), Occludin Rabbit Polyclonal Antibody (AF7644), IL-1β Mouse Monoclonal Antibody (AG2258), E-Cadherin Rabbit Monoclonal Antibody (AG1544), and SNAIL Rabbit Polyclonal Antibody (AF8013). IL-6 (Catalog Number: ab259341), IL-1β (Catalog Number: ab254360), NLRP3 (Catalog Number: ab263899), Vimentin (Catalog Number: ab92547), MMP-9 (Catalog Number: ab76003), HIF-1α (Catalog Number: ab179483), TWIST (Catalog Number: ab50887), ASC (Catalog Number: ab309497), and GSDMD (Catalog Number: ab239377) were purchased from Abcam (UK). The Human IL-6 ELISA Kit (PI330), Human IL-1β ELISA Kit (PI305), and Mouse IL-1β ELISA Kit (PI301) were purchased from Beyotime. FBS (Fetal Bovine Serum), BSA, secondary antibodies, and the BCA Kit were obtained from the Solarbio Company. Dimethyl sulfoxide (DMSO) was sourced from Sigma-Aldrich. Paraformaldehyde and cell culture plates were purchased from Beyotime Co., Ltd. Western blot and IP cell lysis buffers were purchased from Beyotime (Shanghai, China) Company.

Cell source and cell culture

Human hepatocytes (THLE-2) were acquired from Shanghai Jingqi Company, while AML12 (SCSP-550) cells were obtained from The Cell Bank of Type Culture Collection. The liver cancer cell lines, including MHCC97-H (SCSP-5092), Huh-7 (SCSP-526), and SNU-182 (SCSP-5047), were also procured from The Cell Bank of Type Culture Collection. The cells were cultured in Dulbecco's Modified Eagle Medium (DMEM), supplemented with 10% FBS, penicillin (100 U/mL), and streptomycin (100 μg/mL).

Cell treatment with TCBPA

Based on previous reports of TCBPA concentrations in human serum and the environment [15], we selected TCBPA concentrations of 0.1, 0.5, and 1.0 μmol/L for in vitro experiments in the current study.

Western blot assay

Following TCBPA treatment, cells or tissues were washed twice with PBS. Collected cells were transferred to 1.5 mL EP tubes and centri-

fuged at 4°C for 10 minutes. Subsequently, the cells were lysed using lysis buffer and subjected to ultrasonic treatment to disrupt the cells. Following lysis, the cells were placed on ice and incubated for 40 minutes. The protein samples underwent separation by electrophoresis, and subsequently transferred to PVDF membranes. The PVDF membranes were blocked with 10% milk powder and incubated for 1.5 hours. PVDF membranes were washed twice, overlayed with primary antibody solutions, and incubated overnight at 4°C. After primary staining, the PVDF membranes were washed thrice with TBST, secondary antibodies were overlayed, and blots were incubated for 90 minutes. The PVDF membranes were then exposed using an ECL kit. ImageJ was utilized for densitometry analysis of protein bands.

Cell viability assay (MTT)

Cells were counted and seeded into 96-well plates (cell density was adjusted to 1.2×10^4 cells/well, with three replicate wells set up simultaneously). The cells were treated with TCBPA at specified time points. Subsequently, the cells were washed three times with PBS, and MTT solution was added to cell cultures and incubated for 4 hours. After incubation, DMSO was added and incubated for 5 minutes. Absorbance was measured using a microplate reader at a wavelength of 570 nm.

Indirect immunofluorescence

Cells were plated on slides in 6-well plates at a density of 40,000 cells per well. Following TCBPA treatment, the cells were washed three times with PBS. The cell samples were fixed by adding 200 µl of 4% paraformaldehyde for 20 minutes at room temperature. After washing the cells three times with PBS, cells were permeabilized with 200 µl of 0.1% TritonX-100 for 20 minutes and washed thrice with PBS. Slides were blocked with 5% serum for 1 hour in a humidified chamber. Nuclei were stained with Hoechst for 10 minutes in the dark. After washing with 0.01% TritonX, slides were imaged with a laser scanning confocal microscope (CLSM).

EdU cell proliferation assay

Cells were seeded in a culture plate at a density of 2×10^4 cells. Twenty-four hours post-seeding, TCBPA was administered for specified durations. EdU solution (50 µM) was then added to the cells and incubated at room temperature for 2 hours. Cells were then washed with PBS and fixed with paraformaldehyde, followed by three PBS washes. Cells were permeabilized with 0.5% TritonX for 10 minutes. Apollo staining solution was added to the cell samples. After washing the cells, DNA was stained with Hoechst solution for 30 minutes in the dark. Following three PBS washes, cells were viewed under a fluorescence microscope.

Detection of cell death (TUNEL)

Cells were plated in 24-well culture plates. After TCBPA treatment, the culture medium was removed. Cells were stained using a TUNEL staining kit as per the manufacturer's protocol. Cells displaying both DAPI and TUNEL staining were identified as positive. Cell samples were analyzed using a laser scanning confocal microscope.

Animal studies

All animal studies were approved by the Institutional Animal Care and Use Committee of Zhejiang Chinese Medical University. C57 mice were sourced from Hua-Fu-Kang Bioscience Co., LTD. In brief, female BALB/c mice were randomly divided into four groups (8 mice per group). Animals were treated with TCBPA (5, 25, or 50 mg/kg) or corn oil (as a control) for 21 days by gavage [7]. Following treatment, blood and tissue samples were collected for further analysis.

Hematoxylin-eosin staining

Liver tissues were fixed in 4% paraformaldehyde, dehydrated in ethanol, and cleared in xylene before being embedded in paraffin. The liver tissues were then sequentially sectioned. Sections underwent dewaxing, rehydration, and staining with hematoxylin and eosin as per the manufacturer's instructions. The stained sections were observed under a microscope.

Immunofluorescence staining

Tissue sections were blocked with 5% BSA for 30 minutes at room temperature. Sections were then incubated with primary antibody solutions in a humidified chamber at 4°C overnight. The next day, sections were incubated at

room temperature for 30 minutes and washed with PBS (3×5 min). Fluorescently labeled secondary antibodies were applied and incubated in a humidified chamber at room temperature for 2 hours. After washing with PBS (3×5 min), the tissue samples were examined under a laser scanning confocal microscope.

Masson staining

Sections were stained with Weigert's hematoxylin for 8 minutes. The sections were then washed three times with distilled water. Sections were treated with Masson's Ponceau Acid Fuchsin for 10 minutes. Following treatment with 2% glacial acetic acid solution, the sections were incubated in 1% phosphomolybdic acid solution for 5 minutes. Sections were then directly treated with aniline blue for 5 minutes. After treatment with 2% glacial acetic acid solution, the sections were treated with alcohol and xylene and sealed with neutral gum.

Detection of serum inflammatory cytokines by ELISA

ELISA assays were performed according to the manufacturer's instructions. Briefly, the ELISA kits were left at room temperature for 1 hour before starting the experiment. Samples were added to the ELISA plate and incubated at 37°C for 60 minutes. After washing, reagents were added sequentially as per the manufacturer's protocol.

LDH release assay

Lactate dehydrogenase (LDH), which is abundant in the cytoplasm and retained within the cell under normal conditions, can be released upon cell damage or death. Thus, the LDH activity in the cell culture medium is proportional to the number of dead cells. After treating cells with TCBPA, the supernatant was collected for LDH measurement using an LDH kit (LDH Release Kit Cat no. C0017).

Statistical analysis

Experimental data are presented as mean ± standard deviation (Mean ± SD). The T-test was used for comparisons between two groups, while one-way analysis of variance (One-way ANOVA) was applied to analyze data from three or more groups. GraphPad Prism 8.0 software was used for statistical analysis and plotting. $P<0.05$ was considered statistically significant.

Results

Effects of TCBPA on the proliferation of liver cancer cells

MTT assays were conducted to assess the impact of TCBPA on the viability of liver cancer cells (MHCC97-H, Huh-7, and SNU-182). As illustrated in **Figure 1A**, TCBPA promoted liver cancer cell proliferation at low concentrations (0.1, 0.5, and 1 μM) compared to the control (NC) group. Time-dependent studies indicated that TCBPA (0.1, 0.5, and 1 μM) promoted liver cancer cell proliferation (**Figure 1A**, left panel). However, this proliferation-promoting effect of TCBPA was limited in the SNU-182 cell model (Supplementary Figure 1). We also observed that higher concentrations of TCBPA (10 and 20 μM) significantly inhibited liver cancer cell proliferation (**Figure 1A**, right panel). Consequently, the concentrations of 0.01-0.1 and 1 μM were selected for subsequent experiments.

Furthermore, we utilized the EdU cell proliferation assay to examine the effects of TCBPA on liver cancer cell proliferation. The results demonstrated an increase in the EdU signal in the TCBPA treatment group compared to the NC group (**Figure 1B**), suggesting that TCBPA promotes liver cancer cell proliferation.

We evaluated protein molecules and signaling pathways associated with liver cell proliferation to explore the impact of TCBPA on liver cancer cells. Western blot was performed to detect changes in apoptosis-related proteins, revealing decreased BAX expression and increased Bcl-2 expression (**Figure 1C**). Cell cycle proteins, Cyclin D1 and Cyclin E, were also analyzed and demonstrated increased expression under TCBPA treatment (**Figure 1D**). Marker molecules for cell proliferation were assessed, demonstrating upregulated expression of PCNA and c-Myc (**Figure 1E**). Additionally, PTEN expression was downregulated under TCBPA treatment (**Figure 1F**).

TCBPA induced inflammatory response in liver cancer cells

We first analyzed the impact of TCBPA on inflammation, revealing that TCBPA can pro-

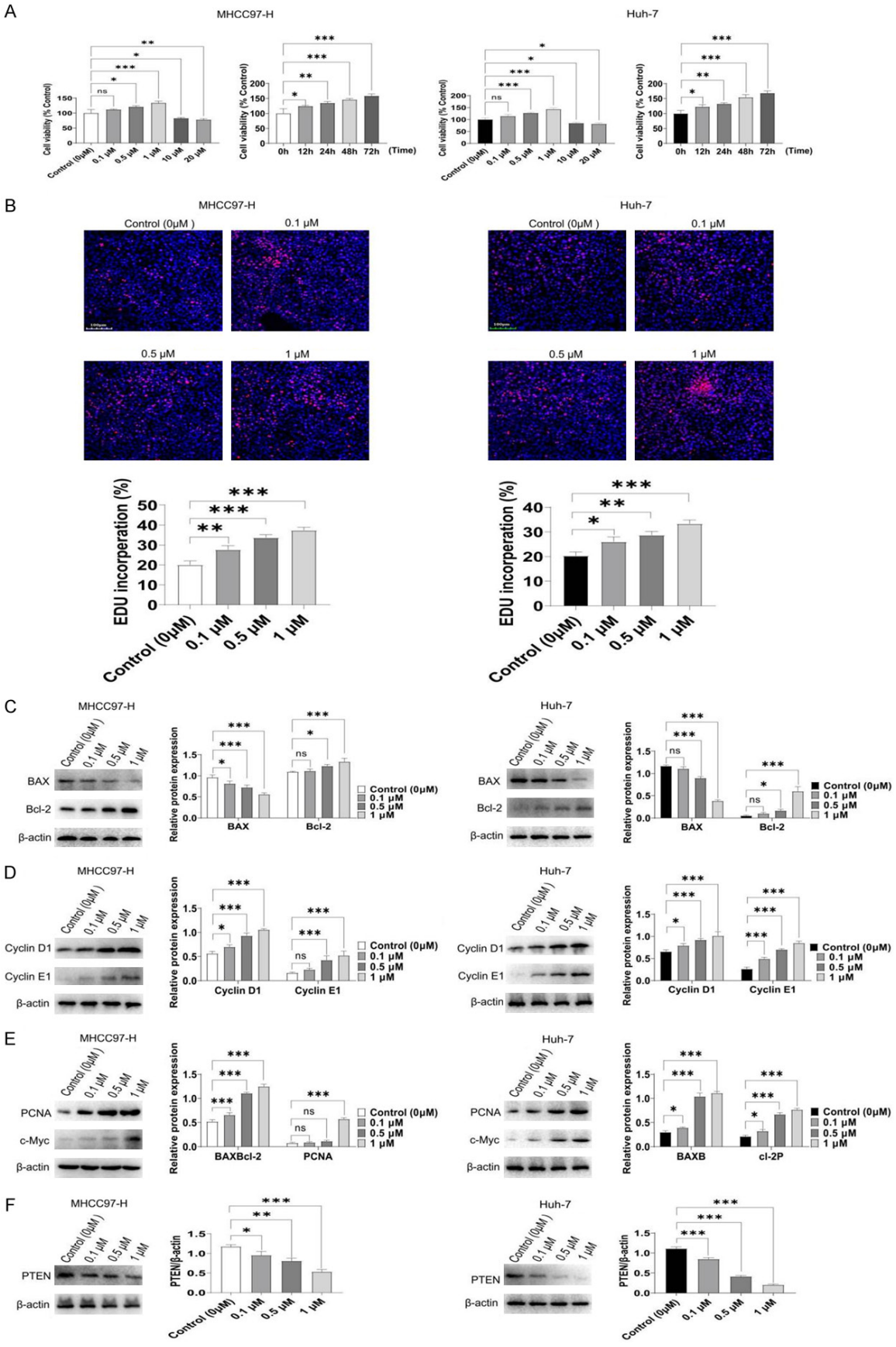
A
MHCC97-H
Huh-7
Cell viability (% Control)
Control (0μM)
0.1 μM
0.5 μM
1 μM
10 μM
20 μM
0h
12h
24h
48h
72h
(Time)
B
MHCC97-H
Huh-7
Control (0μM)
0.1 μM
0.5 μM
1 μM
EDU incorporation (%)
C
MHCC97-H
Huh-7
BAX
Bcl-2
β-actin
Relative protein expression
D
Cyclin D1
Cyclin E1
E
PCNA
c-Myc
BAXBcl-2
BAXB
cl-2P
F
PTEN
PTEN/β-actin

Figure 1. Effects of TCBPA on the proliferation of liver cancer cells. A. MTT analysis of the impact of TCBPA on the proliferative capacity of MHCC97-H and Huh-7. B. Analysis of cell proliferation by EdU assay under TCBPA treatment. C. Effects of TCBPA on Bax and Bcl-2 expression. D. Effects of TCBPA on Cyclin D1 and Cyclin E expression. E. Impact of TCBPA on the expression of PCNA and c-Myc. F. Down-regulation of PTEN expression by TCBPA treatment. $P<0.05$ indicates significance.

mote the expression of inflammatory factors, including IL-6 and IL-1β (**Figure 2A**). Additional experiments demonstrated that TCBPA activates the inflammatory signaling molecule NF-κB. Furthermore, the expression of STAT3, an inflammation-related molecule, was also upregulated (**Figure 2B**).

We then asked the question of how TCBPA activates NF-κB. As an exogenous chemical molecule (also known as a danger-associated molecular pattern, DAMP), TCBPA induces cellular stress upon stimulation. Consequently, endogenous DAMPs are released and sensed by a pattern recognition receptor called NLRP3, which forms an inflammasome. Analysis of NLRP3 expression showed a strong induction by TCBPA, while the expression levels of NLRP1 and AIM2 remained unchanged (**Figure 2C**). To confirm whether NLRP3 is essential for NF-κB activation, an NLRP3 inhibitor, MCC950, was used; its inhibitory effect on NLRP3 was verified in Supplementary Figure 3. The results showed that NF-κB activation was significantly suppressed (**Figure 2D**). Simultaneously, liver cancer cell proliferation was also significantly inhibited when either NLRP3 or NF-κB was suppressed (**Figure 2E**). Additionally, HCC cells barely expressed GSDMD under these experimental conditions (Supplementary Figure 2).

Impact of TCBPA on endothelial-to-mesenchymal transition (EMT) in liver cancer cells

We examined the effects of TCBPA on EMT in liver cancer cells by evaluating the expression of Snail, E-cadherin, Claudin-1, Vimentin, MMP-9, HIF-1α, TWIST, Occludin, and ZO-1 under TCBPA treatment. Western blot results indicated a significant increase in the expression of Vimentin, HIF-1α, Snail, and TWIST. Conversely, the expression of E-cadherin, Claudin-1, ZO-1, and Occludin was significantly downregulated. Moreover, MMP-9 expression levels were significantly elevated (**Figure 3**).

Impact of TCBPA on normal liver cell proliferation

Two normal liver cell lines (THLE-2 and AML12) were used to evaluate the toxicological effects of TCBPA exposure in MTT assays, where the effects of TCBPA on liver cell viability were assessed. As shown in **Figure 4A**, we observed that TCBPA could inhibit the proliferation of normal liver cells at concentrations of 0.01, 0.1, and 1 μM compared to the control group. Furthermore, time course experiments yielded similar results (**Figure 4B**).

Further investigation into the influence of TCBPA on liver cell inflammation revealed a significant increase in the expression levels of IL-1β and IL-6 (**Figure 4C**). Additional experiments indicated that TCBPA could activate NLRP3, ASC, GASDMD, and upregulate Caspase 1 expression (**Figure 4D**). Cell death was assessed by PI staining (**Figure 4E**). Pyroptosis is characterized by the release of DAMPs including ATP and HMGB1. Our subsequent experiments found significantly increased ATP and HMGB1 levels in the cell supernatant compared to the control group (**Figure 4F**). When cells were treated with the pyroptosis inhibitor, VX765, cell death was significantly alleviated as evidenced by ATP and HMGB1 levels in the cell culture supernatant (**Figure 4G**). These data suggest that TCBPA induces cell death primarily through pyroptosis.

In vivo effects of TCBPA on liver tissue

H&E staining revealed that TCBPA disrupted hepatic cord structures, causing significant hepatocyte swelling and visible intracellular lipid vacuoles. TCBPA-treated mouse liver exhibited extensive hepatocyte vacuolar degeneration, hepatocyte lipid deposition, and inflammatory cell infiltration in the portal area. In contrast, the control group displayed neatly arranged hepatic cord structures, clear lobular architecture, rich cytoplasm, and centrally located nuclei (**Figure 5A**). Indirect immunofluorescence was used to evaluate the impact of TCBPA on inflammation, showing a significant increase in liver inflammation levels compared to the control group (**Figure 5B**). Moreover, liver fibrosis staining indicated evident fibrosis in the TCBPA treatment group compared to the control group (**Figure 5C**). Furthermore, Western blotting indicated that liver cell cycle proteins,

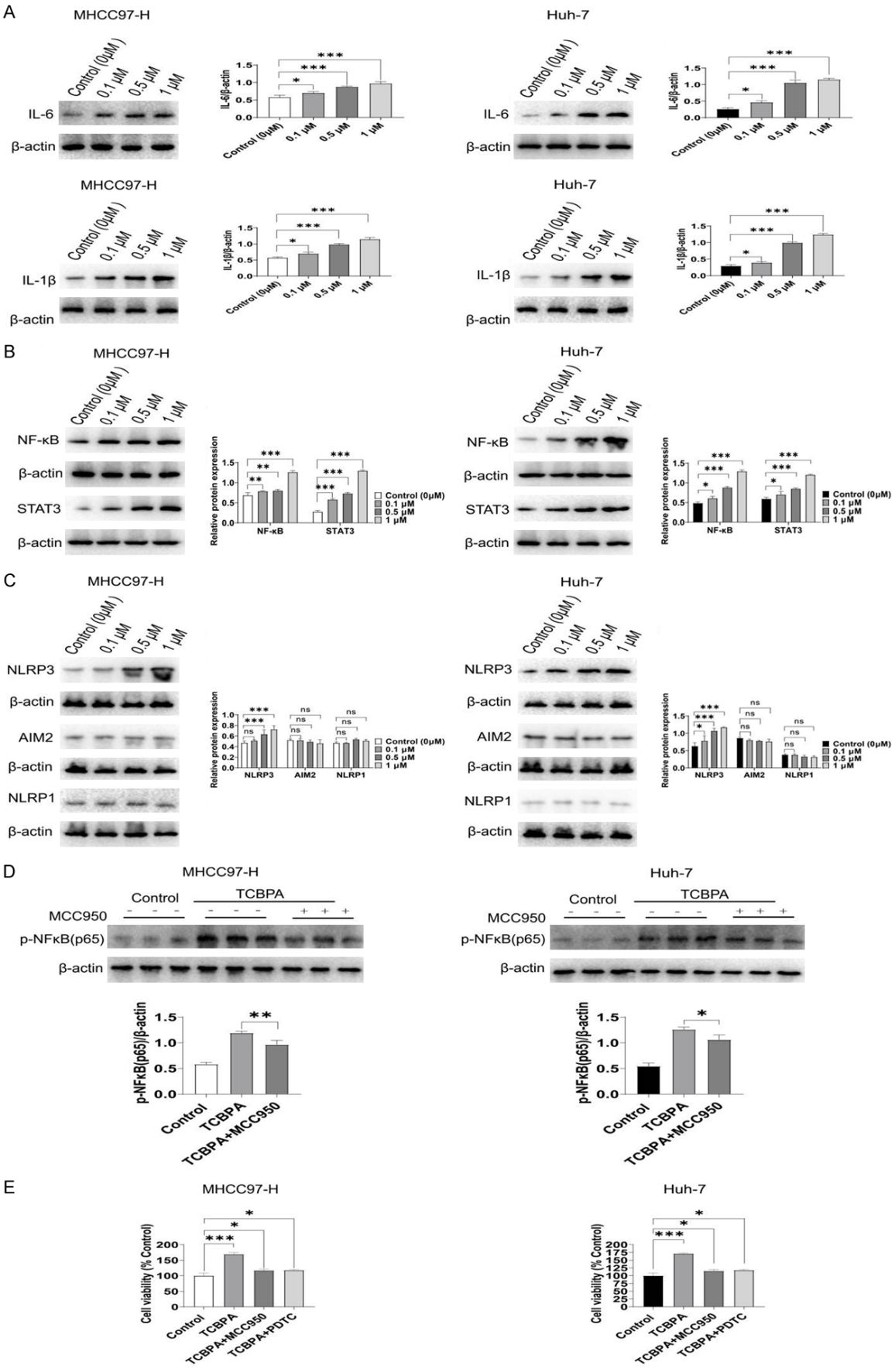
A
MHCC97-H
Huh-7
Control (0μM)
0.1 μM
0.5 μM
1 μM
IL-6
β-actin
IL-6/β-actin
IL-1β
IL-1β/β-actin
B
NF-κB
STAT3
Relative protein expression
Control (0μM)
0.1 μM
0.5 μM
1 μM
C
NLRP3
AIM2
NLRP1
ns
D
Control
TCBPA
MCC950
p-NFκB(p65)
p-NFκB(p65)/β-actin
TCBPA+MCC950
E
Cell viability (% Control)
TCBPA+PDTC

Figure 2. TCBPA-induced inflammatory response in liver cancer cells. A. TCBPA promotes the expression of inflammatory factors (IL-6 and IL-1β). B. TCBPA activated NF-κB and STAT3. C. Effects of TCBPA on NLRP3, NLRP1, and AIM2 expression. D. NLRP3 is essential for NF-κB activation. E. Liver cancer cell proliferation was inhibited by NF-κB suppression. P<0.05 indicates significance.

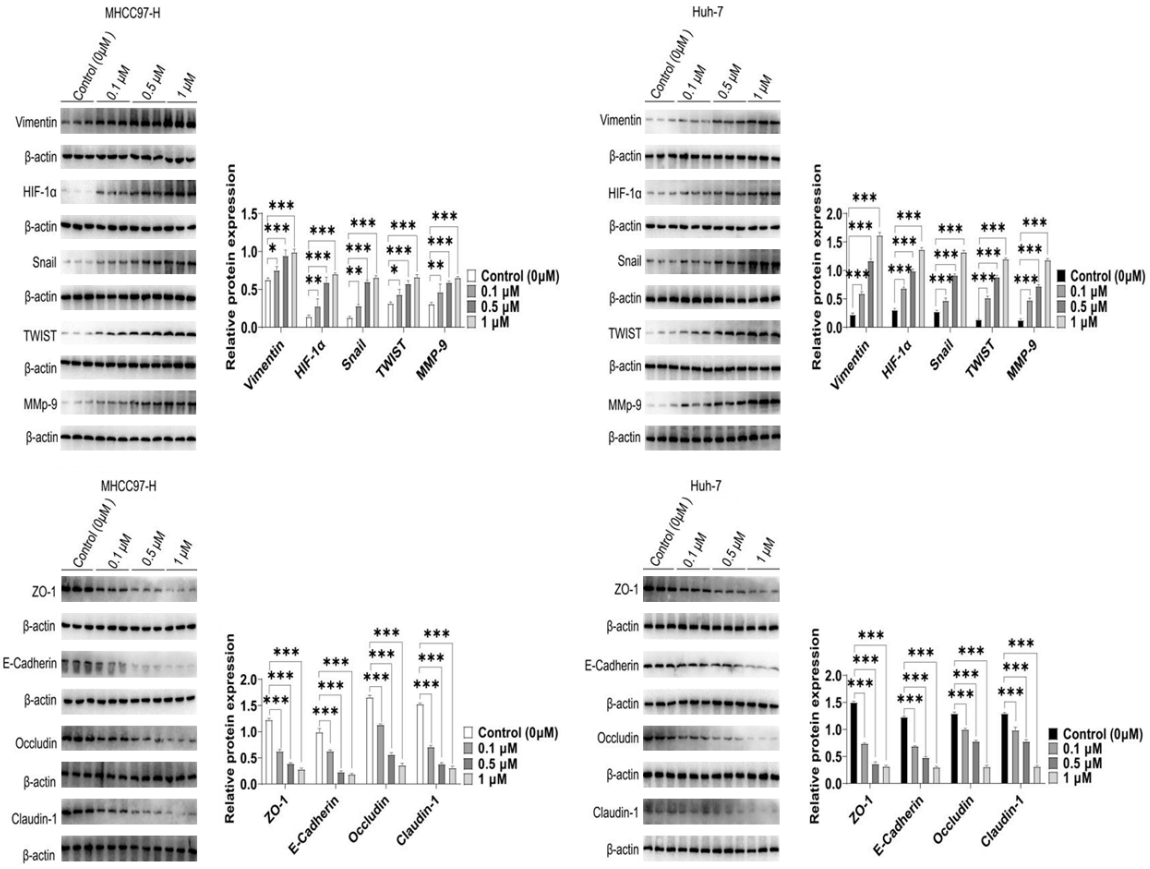

Figure 3. Impact of TCBPA on EMT in liver cancer cells. Western blot analysis of the effects of TCBPA on the expression of Snail, E-cadherin, Claudin-1, Vimentin, MMP-9, HIF-1α, TWIST, Occludin and ZO-1. P<0.05 indicates significance.

Cyclin D1 and Cyclin E, were significantly downregulated (**Figure 5D**). Pyroptosis-related markers NLRP3, GSDMD, and cleaved Caspase-1 were also found to be upregulated (**Figure 5E**).

In summary, we have consolidated the findings of the current study, as illustrated in **Figure 6**, which highlights the differential effects of TCBPA on liver cancer cells and liver cells: Two sides of the coin.

Discussion

TCBPA and TBBPS have recently been widely used as alternatives to TBBPA [16]. The manufacturing, storage, and application of materials containing TCBPA can lead to its release, resulting in its occurrence and accumulation in the environment. Consequently, TCBPA residues are frequently detected in air, water, soil, dust, sludge, food, and aquatic organisms, attracting widespread attention from environmental scientists [17]. Studies have indicated that long-term exposure to TCBPA can result in biological toxicity, potentially causing endocrine disruptions in aquatic organisms and neurotoxic and cytotoxic effects in humans. TCBPA can persist as an organic pollutant in the environment such as in water, the atmosphere, sediments, and also in organisms. Due to its high lipophilicity, cytotoxicity, and immunotoxicity, TCBPA poses significant risks for human health and the environment [18]. To date, potential risks of TCBPA

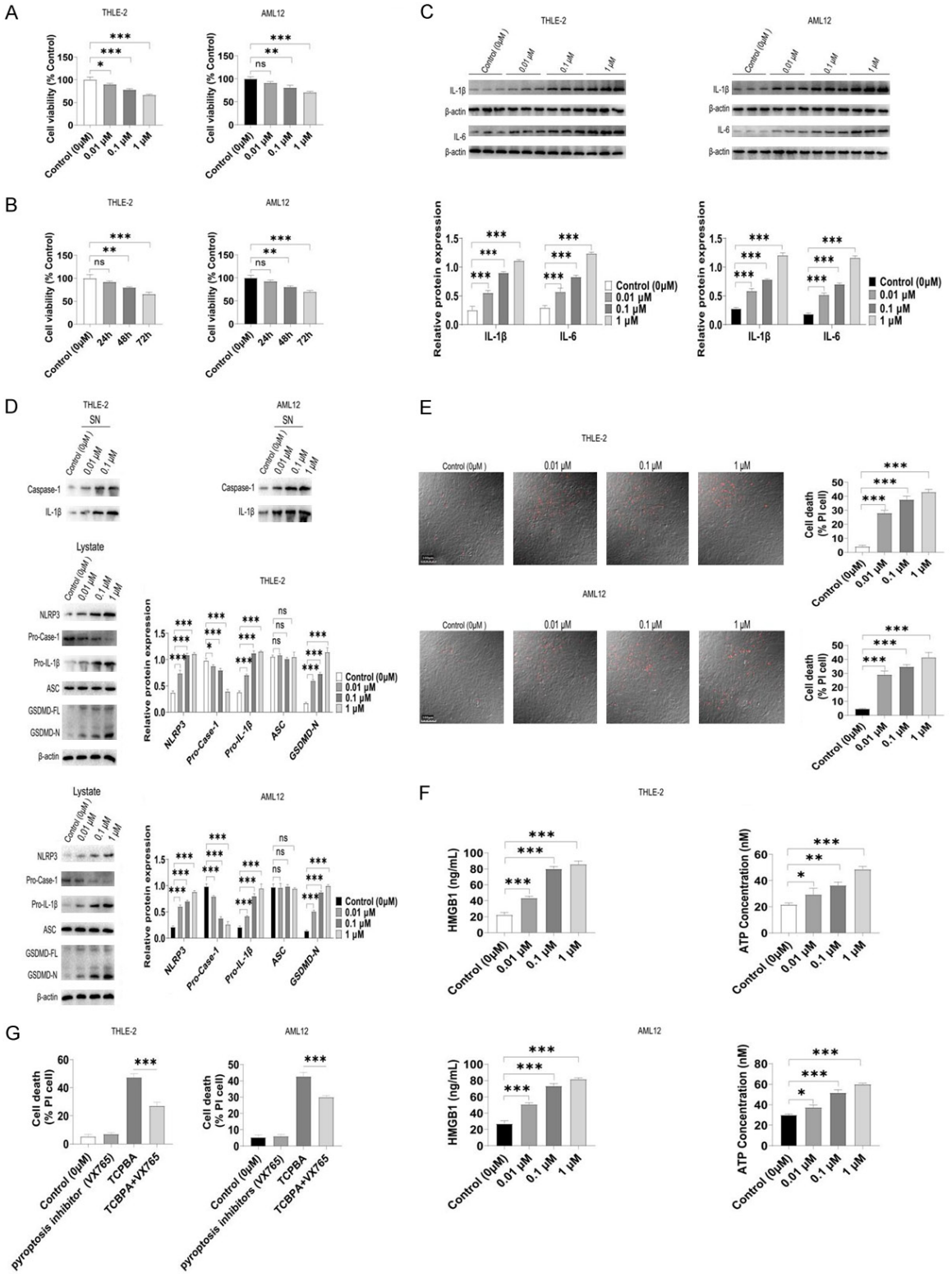

Figure 4. TCBPA-induced pyroptosis in THLE-2 and AML12 cells. A. TCBPA inhibited the proliferation of normal liver cells. B. TCBPA suppressed normal liver cell proliferation at different time points. C. TCBPA promoted the expression of IL-1β, and IL-6. D. TCBPA activated NLRP3, ASC, GASDMD, and Caspase 1. E. Assessment of TCBPA-induced liver cell death by PI staining. F. Effects of TCBPA on HMGB1 and ATP release. G. Cell death was alleviated by pyroptosis inhibition. P<0.05 indicates significance.

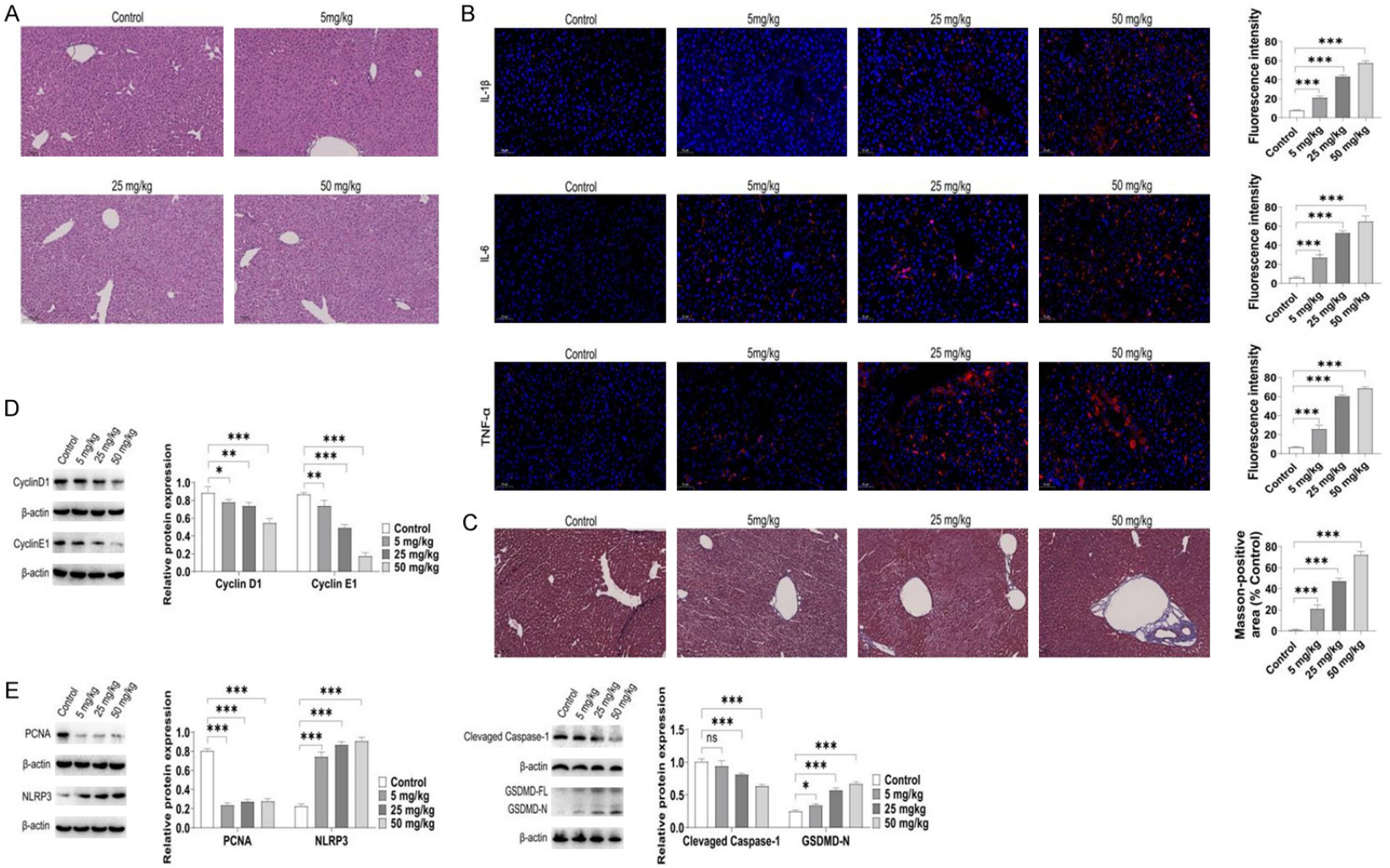

Figure 5. Effects of TCBPA on liver tissue *in vivo*. A. H&E staining of the impact of TCBPA on the liver. B. Effects of TCBPA on inflammation. C. TCBPA-induced liver fibrosis. D. Effects of TCBPA on Cyclin protein expression. E. Impact of TCBPA on the expression of pyroptosis-related molecules. P<0.05 indicates significance.

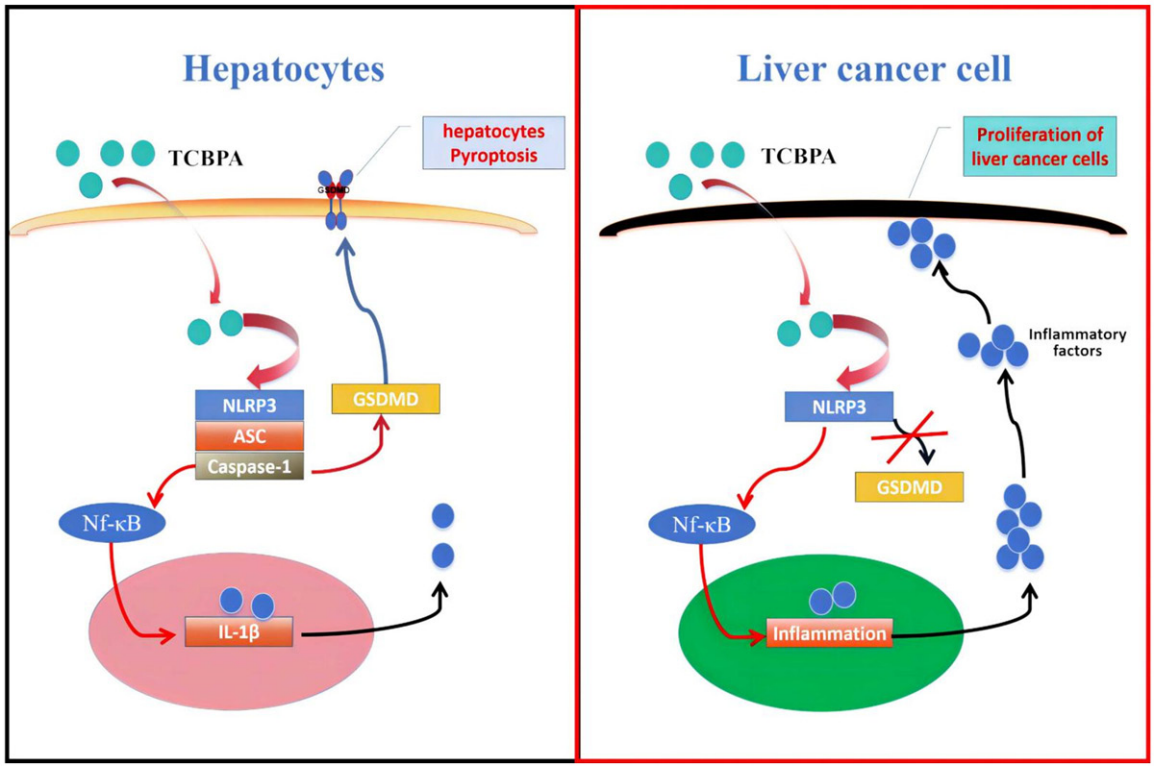

Figure 6. Molecular mechanisms of TCBPA leading to two sides of the same coin.

as a safe alternative to TBBPA remain largely unknown. In the current study, we systematically examined the effects of TCBPA on normal liver cells and hepatoma cell lines. Our results showed that TCBPA was able to promote the proliferation of hepatoma cells while inhibiting the growth of normal liver cells.

In this work, we investigated the effect of TCBPA on liver cancer cells. Interestingly, we found that TCBPA treatment promoted the proliferation of liver cancer cells through a series of biochemical analyses. We investigated the mechanisms behind the promotion of liver cancer cell proliferation by TCBPA and potential molecules involved. TCBPA can induce a stress response in cells, leading to damage. In response to stress, cells will produce corresponding damage-related signaling molecules, such as DAMP, an endogenous molecule released from damaged cells. DAMP can activate the innate immune system by interacting with pattern recognition receptors (PRR) [19]. Consequently, we examined the inflammatory response of tumor cells under TCBPA treatment, finding a significant increases in the expression levels of IL-6 and IL-1β. Next, our evaluation revealed that TCBPA activated the expression of inflammatory signaling molecules NF-κB and STAT3. Studies have shown that there is crosstalk between STAT3 and NF-κB expression. Studies have shown a cross-talk between STAT3 and NF-κB expression, both of which regulate a wide range of downstream genes involved in cell proliferation, survival, stress responses, and immune functions, with some target genes overlapping [20].

Previous research has demonstrated a close association between inflammation and tumorigenesis [21]. Inflammatory factors can promote the initiation and progression of tumors. For instance, the inflammatory factor IL-6 can stimulate the growth of tumor cells [22]. The current study suggests that TCBPA may promote the proliferation of liver tumor cells through the action of inflammatory factors. TCBPA has also been shown to enhance the proliferation of breast cancer cells in a cellular breast cancer model [23]. It remains puzzling why NLRP3 did not induce pyroptosis in liver cancer cell models, as it is closely related to the expression of the key molecule GSDMD that mediates pyroptosis. Many tumor cells express GSDMD at low

levels, reducing the likelihood of pyroptosis. In the liver cancer cells examined in this study, GSDMD expression was not detected.

While previous studies indicated that NF-κB can activate NLRP3, our findings suggest that NLRP3 activation is necessary for NF-κB activation, consistent with prior reports. In contrast, Kinoshita et al. found that NLRP3 activates NF-κB during sterile inflammation [24]. In contrast, TCBPA inhibits the proliferation of normal liver cells. When liver cells are exposed to TCBPA, they undergo stress, leading to the production of DAMPs. The receptors that recognize these DAMPs are known as pattern recognition receptors (PRRs), which are divided into four main categories: Toll-like receptors (TLRs), Retinoic acid-inducible gene (RIG)-I-like receptors (RLRs), NOD-like receptors (NLRs), and C-type lectin receptors (CLRs), which are widely expressed in immune cells. Currently, there are three main types of cell death: apoptosis, necroptosis, and pyroptosis [25, 26]. In this work, we found that TCBPA activated NLRP3-induced cell pyroptosis by analyzing a series of biomarkers. We discovered that TCBPA induced pyroptosis in normal liver cells, but not in hepatoma cells.

In summary, this study evaluated the impact of TCBPA on liver cells and liver cancer cells, demonstrating that TCBPA exposure promotes the malignant progression of liver cancer cells while leading to damage of normal liver cells. The current study will provide valuable information for assessing the potential health impacts of TCBPA.

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Disclosure of conflict of interest

None.

Abbreviations

BFRs, Brominated flame retardants; TBBPA, tetrabromobisphenol A; DMEM, Dulbecco's Modified Eagle Medium.

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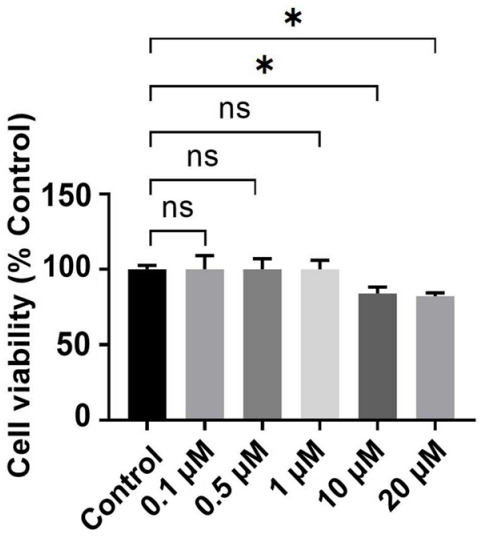
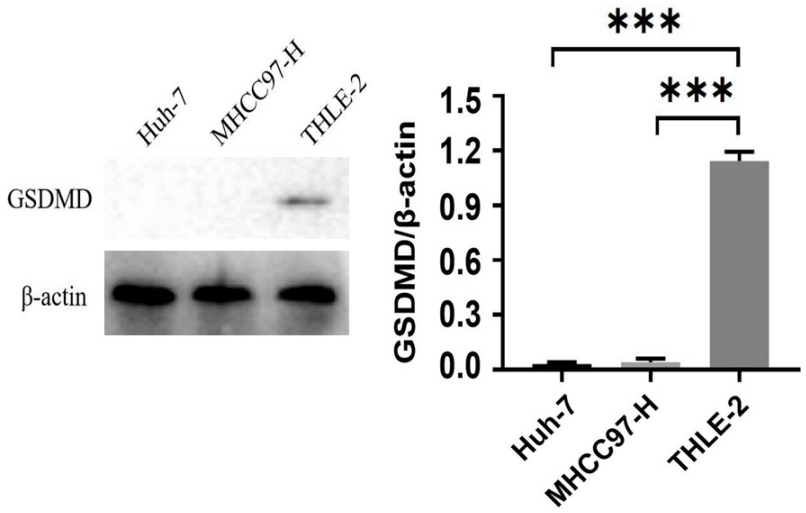
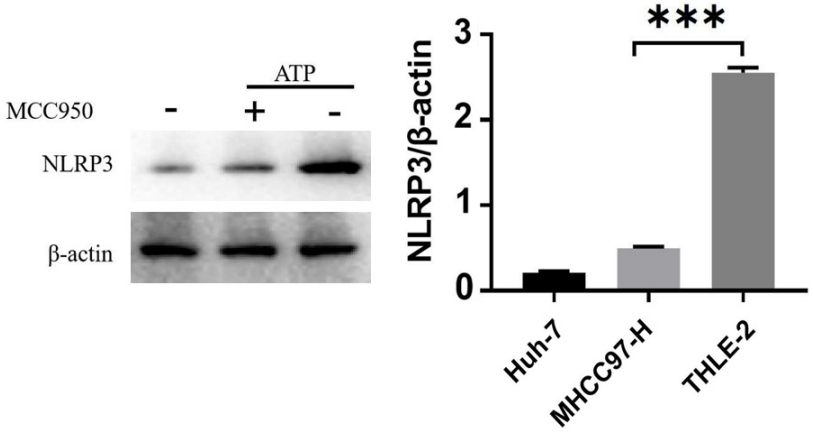


Supplementary Figure 1. Effects of TCBPA on the proliferation of SNU-182 cell.



Supplementary Figure 2. Inhibitory effect on NLRP3 inhibitor (MCC950) was evaluated.



Supplementary Figure 3. HCC cells barely expressed GSDMD.