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Original Article

Dual opposing effects of TBBPS/TCBPA on cervical cell proliferation: suppression in normal cells versus promotion in cancer via the cGAS-STING pathway

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Abstract: TBBPS and TCBPA are increasingly used as alternatives to tetrabromobisphenol A (TBBPA) and have been detected in various environmental samples. Moreover, they have been widely detected in human biological matrices such as blood, milk, *et al*. In view of this, it is particularly urgent to comprehensively evaluate the toxicological properties of TBBPS/TCBPA. In this study, in vitro experiments were carried out with cervical cancer cell models and normal cervical cell models. Comprehensive biochemical experiments were conducted to examine the effects of TBBPS and TCBPA on cervical cancer cells. Cell proliferation assays with CCK8 and EdU demonstrated that TBBPS and TCBPA enhance cervical cancer cell proliferation and enhance the expression of proliferation-related molecules. Subsequent research demonstrated that TBBPS/TCBPA enhance the secretion of inflammatory factors in cervical cancer cells, influencing cell proliferation. In the cervical epithelial cell model, our study revealed that TBBPS/TCBPA suppressed the proliferation of normal cervical epithelial cells. Mechanistic studies revealed that TBBPS/TCBPA treatment enhanced ds-DNA release, thereby activating the cGAS-STING signaling pathway and inhibiting cell proliferation. This study concludes that TBBPS/TCBPA exhibit dual effects by promoting cervical cancer cell growth while inhibiting normal cervical cell proliferation, which was mediated through the regulation of the inflammatory response. This study's findings will serve as a foundation for evaluating the potential health risks posed by TCBPA/TBBPS exposure.

Keywords: TBBPS, TCBPA, cervical cancer, cervical cells, cGAS-STING

Introduction

Cervical cancer ranks as the fourth most prevalent malignant tumor in women and has the highest mortality rate among female cancers, significantly threatening women's health and safety, thus earning it the moniker 'female killer [1]. Epidemiological data indicate that cervical cancer causes more than one million deaths each year. Cervical cancer primarily develops at the cervicovaginal junction. Epidemiological data show that three main categories of risk factors affect cervical cancer [2, 3]. The first category is biological factors, with cervical cancer development closely linked to various pathogens, including viral infections such as HPV and HSV. The second category is behavioral risk factors, such as premature sexual intercourse. The third category is genetic susceptibility [4, 5]. Recent studies indicate that environmental factors, including chemical pollutants and radiation, contribute to the development of cervical cancer. These risk factors interact with each other and significantly contribute to cervical cancer development [6, 7]. The theory of environmental carcinogenesis states that approximately 80%-90% of the causes of human malignant tumors are attributed to environmental factors.

At present, numerous environmental factors implicated in tumorigenesis can be categorized

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into three types by their properties: chemical, physical, and biological, among which chemical factors predominate. Tetrabromobisphenol-type compounds and their derivatives are widely used brominated flame retardants, extensively utilized in building materials, textiles, and electronic equipment. Brominated flame retardants (BFRs), including tetrabromobisphenol compounds, have been commonly detected in human blood [8]. Tetrabromobisphenol A (TBBPA) and its derivatives are the predominant brominated flame retardants in commercial products. However, in recent years, due to growing toxicological concerns about TBBPA, it has been gradually banned. Currently, TBBPS and TCBPA are more widely used. TBBPS and TCBPA are widely used as alternatives to TBBPA in flame-retardant applications, added to building materials, textiles, plastic products, etc., to improve the fire-resistance performance of these materials [9]. The relationship between TBBPS/TCBPA and cervical cancer remains unclear, posing an unresolved scientific question.

Cervical cancer progression is closely linked to inflammation. Inflammation caused by viral infections is an important factor promoting tumor progression [10]. Infiltrating immune cells and the cytokines, chemokines, and growth factors they secrete significantly promote malignant phenotypes of cervical cancer. Inflammatory factors such as IL-6 and IL-1β are significantly associated with tumor initiation and progression [11]. Research indicates that cervical cancer cells secrete IL-6 and IL-1β, which promote the proliferation of cancer cells. Recent studies indicate that the cGAS-STING signaling pathway is crucial in detecting cytoplasmic abnormal DNA accumulation and tumor development, and targeting this pathway may mitigate tumor progression [12]. The cGAS-STING signaling pathway influences tumor development by mediating inflammation [13].

Here, we used the HeLa cells and SiHa cells as in vitro models to explore the toxicological effects of TCBPA/TBBPS. We found that TCBPA/TBBPS exposure promotes the proliferation of cervical cancer cells. In contrast, they inhibit the proliferation of normal cervical epithelial cells. This study's findings will serve as a foundation for evaluating the potential health risks posed by TCBPA/TBBPS exposure.

Materials and methods

Chemicals and antibodies

DMEM medium, PBS and DMSO were from Solarbio company (Beijing, China). The following materials were sourced from Abcam (UK): Anti-IL-1β, anti-IL-6, anti-TNFα, polyvinylidene fluoride (PVDF), anti-STING, anti-TBK1, and nuclear factor kappa-B (NF-κB). Bovine serum albumin, goat anti-rabbit IgG and DAPI fluorescent dye were from Solarbio (Beijing, China). The Cell Counting Kit, trypsin cell digestion solution, protease inhibitor cocktail, skim milk powder, EDTA, bovine serum albumin (BSA), fetal bovine serum (FBS), hematoxylin and eosin, antibody elution solution, and ECL chemiluminescence detection kit were sourced from Beyotime Biotechnology (Shanghai, China).

Cell culture

HeLa cells and SiHa cells were purchased from the National Collection of Authenticated Cell Cultures. Cells were cultured in DMEM medium with 10% FBS at 37°C with 5% CO_2.

Quantitative analysis of proinflammatory cytokines by ELISA

Post-treatment, the supernatant from HeLa and SiHa cell cultures was collected into a centrifuge tube and centrifuged at 1000 RPM for 20 minutes at 4°C to remove cell debris. The supernatant was collected and used as samples, which were incubated in each well for 90 minutes. Subsequently, the cells were washed three times, followed by adding 100 μl of enzyme-conjugated solution to each well for 30 minutes. Following sample washing, 80 μl of TMB was added to each well and allowed to react in the dark for approximately 10 minutes. The reaction was terminated by adding stop solution to each well, followed by measuring the absorbance at 450 nm using an ELISA reader.

Western-blot analyses

Cells were exposed to TBBPS/TCBPA for specified time intervals. Cell samples were washed with PBS, scraped into 1.5 ml EP tubes, and centrifuged at 1500 RPM for 10 minutes at 4°C. An appropriate amount of lysate was added to the EP tubes, which were then placed

on ice for 10 minutes. Samples were sonicated using an ultrasonic cell disruptor (3 seconds each time). Cell lysates were subjected to SDS-PAGE analysis. Proteins were electrophoretically transferred to a PVDF membrane. The PVDF membrane was blocked with 5% non-fat dry milk and shaken for 120 minutes. After washing three times with TBST, the PVDF membrane was incubated with primary antibodies at 4°C overnight. Unbound primary antibodies were washed off by TBST washes. The membrane was washed with TBST and incubated with secondary antibodies for 2 hours. Protein bands were then detected using ECL solution.

Cell viability test (CCK8)

Cell viability was evaluated using the CCK8 kit according to the manufacturer's instructions to determine the effects of TBBPS/TCBPA. Cells (HeLa and SiHa) were seeded into a 96-well culture plate. After treating the cells with TBBPS/TCBPA, CCK8 solution was added. After incubation for 4 h, the cell samples were tested with a microplate reader.

Immunofluorescence

After TBBPS/TCBPA stimulation, the cells were fixed with 4% PFA at room temperature for 0.5 h. After washing, cells were permeabilized and blocked with 10% normal goat serum for 0.5 h. After washing, the primary antibodies were added and incubated at 4°C for 12 h, followed by incubated with fluorescently labeled secondary antibodies for 2 h. Cell samples were checked by CLSM.

Cell exposure experiment

When the cells reached approximately 70% confluence, cells were challenged with TBBPS/TCBPA (0.01 μM-0.1 μM) for 24 h. TBBPS/TCBPA concentrations were selected based on previous reports [14, 15].

EdU assay for cell proliferation

EdU incorporation assays were performed to assess the effects of TBBPS/TCBPA on cell proliferation. Cervical cancer cells were seeded in a 96-well cell culture plate and exposed to TBBPS/TCBPA. After washing, cells were incubated with EdU solution at 37°C for 120 minutes according to the EdU kit protocol. The cell samples were analyzed using a laser confocal microscope (Olympus, FV3000).

ROS detection

The cells (HeLa and SiHa) were challenged with TBBPS/TCBPA. Cells were then incubated with DCFH-DA probes at 37°C for 0.5 h. After washing, The cells were then examined using a laser confocal microscope (FV3000).

Detection of Mitochondrial Membrane Potential (MMP)

To analyze the effects of TBBPS/TCBPA on MMP, the cells were challenged with TBBPS/TCBPA for 24 h. After washing, the cells were stained with TMRE, the samples were detected with CLSM.

PI staining

After the cells were exposed to TBBPS/TCBPA, cells were stained with the PI. The samples were detected with CLSM.

MitoSOX dyeing

To study the effect of TBBPS/TCBPA on mitochondrial ROS, the cells were exposed to TBBPS/TCBPA for 24 h. After washing, MitoSOX solution was added at 37°C and incubated for 10 min in the dark. After washing, the cell samples were observed using a confocal microscope.

ELISA assays

ELISA assays for IL-1β and IL-6 were performed using commercial kits. Reagents were equilibrated to room temperature for 30 minutes. Test samples and standards were incubated on the ELISA plate at 37°C for 1 hour. After washing, biotin-labeled antibodies were added and incubated at 37°C for 1 hour. Wells were washed three times, incubated with Streptavidin-HRP at 37°C for 30 minutes, washed again, and developed with substrate solution. Absorbance was measured at 450 nm using a microplate reader.

Statistical analysis

The relevant data were collated and analyzed for significant differences using GraphPad Prism 8. A one-way ANOVA was employed to compare data across multiple groups, while a t-test assessed differences between two groups. $P<0.05$ represented a significant difference and was statistically significant.

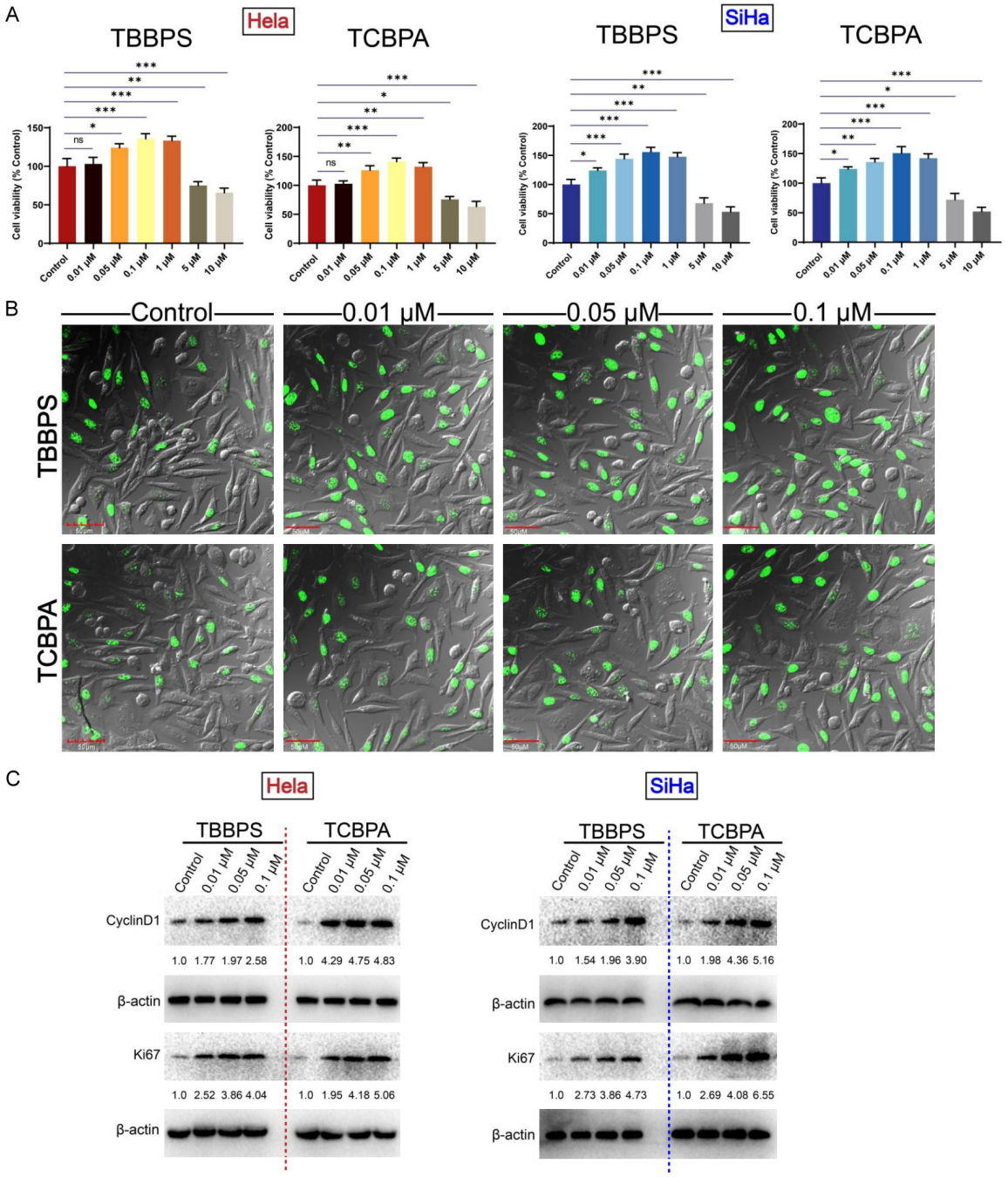

Figure 1. Investigation of TBBPS and TCBPA on cervical cancer cell proliferation. A. Assessment of TCBPA/TBBPS impact on cervical cancer cell proliferation using CCK8 assay. B. Analysis of the effects of TCBPA/TBBPS on cell proliferation by EdU. C. Impact of TCBPA/TBBPS on cell proliferation marker expression. A *p*-value less than 0.05 denotes a statistically significant difference.

Results

Impact of TBBPS/TCBPA on cervical cancer cell activity

CCK8 assays were conducted to assess the impact of TBBPS/TCBPA on cervical cancer cell viability. TBBPS and TCBPA were tested at concentrations of 0.01, 0.05, 0.1, 1, 5, and 10 μM, selected based on reported environmental exposure levels. The findings indicated that low concentrations of TCBPA (0.01-0.1 μM) enhanced the proliferation of HeLa and SiHa cells compared to the control group. However, when the concentration of TBBPS/TCBPA exceeded

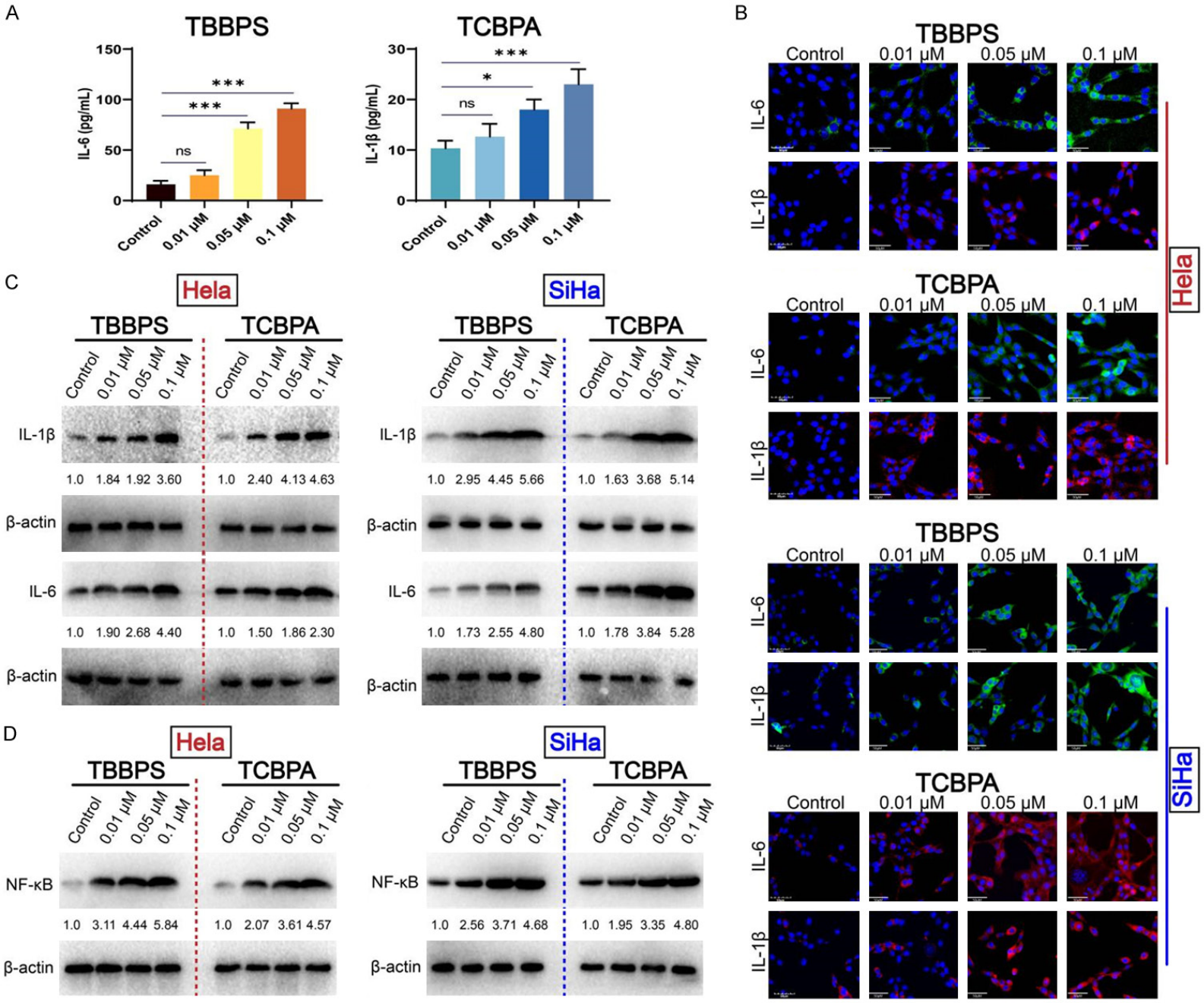
A
TBBPS
IL-6 (pg/mL)
150
100
50
0
ns
***
***
Control
0.01 μM
0.05 μM
0.1 μM
TCBPA
IL-1β (pg/mL)
30
20
10
0
ns
*
***
Control
0.01 μM
0.05 μM
0.1 μM
B
TBBPS
Control
0.01 μM
0.05 μM
0.1 μM
IL-6
IL-1β
Hela
TCBPA
Control
0.01 μM
0.05 μM
0.1 μM
IL-6
IL-1β
Hela
TBBPS
Control
0.01 μM
0.05 μM
0.1 μM
IL-6
IL-1β
SiHa
TCBPA
Control
0.01 μM
0.05 μM
0.1 μM
IL-6
IL-1β
SiHa
C
Hela
TBBPS
TCBPA
Control
0.01 μM
0.05 μM
0.1 μM
IL-1β
1.0 1.84 1.92 3.60
1.0 2.40 4.13 4.63
β-actin
IL-6
1.0 1.90 2.68 4.40
1.0 1.50 1.86 2.30
β-actin
SiHa
TBBPS
TCBPA
IL-1β
1.0 2.95 4.45 5.66
1.0 1.63 3.68 5.14
β-actin
IL-6
1.0 1.73 2.55 4.80
1.0 1.78 3.84 5.28
β-actin
D
Hela
TBBPS
TCBPA
NF-κB
1.0 3.11 4.44 5.84
1.0 2.07 3.61 4.57
β-actin
SiHa
TBBPS
TCBPA
NF-κB
1.0 2.56 3.71 4.68
1.0 1.95 3.35 4.80
β-actin

Figure 2. Impact of TBBPS/TCBPA on inflammatory factor expression. A. IL-6 and IL-1β expression was measured using ELISA. B. Indirect immunofluorescence was used to analyze IL-6 and IL-1β expression. C. Western blot analysis of inflammatory factor expression. D. Impact of TBBPS/TCBPA on NF-κB Expression. A *p*-value less than 0.05 signifies a statistically significant difference.

5 μM, the proliferative capacity of cervical cancer cells was significantly inhibited (**Figure 1A**). Based on these results, we selected 0.01, 0.05, and 0.1 μM (TCBPA and TBBPS) as the concentration range for subsequent experiments. We used the EdU assay to validate the proliferative effects of TBBPS/TCBPA on cervical cancer cells. The experimental findings confirmed that the TBBPS/TCBPA-treated group exhibited significantly more EdU-positive cells compared to the negative control (NC) group, further indicating that TCBPA stimulates cervical cancer cell proliferation (**Figure 1B**). We subsequently evaluated the impact of TBBPS/TCBPA on cell-cycle proteins and proliferation markers. Our findings indicate that TBBPS/TCBPA significantly upregulates CyclinD1 and Ki67 expression levels, suggesting a potential role in enhancing cell proliferation by promoting cell-cycle progression (**Figure 1C**).

TBBPS/TCBPA induced production of IL-6/IL-1β through activation of NF-κB

Research indicates that inflammatory factors like IL-6 and IL-1β contribute to the initiation and progression of cervical cancer [16]. Previous studies have shown that TCBPA/TBBPS induce tumor cells to secrete inflammatory factors. We initially examined the impact of TBBPS/TCBPA on inflammatory factor secretion in cervical cancer. These findings indicated that TBBPS/TCBPA enhanced the expression of inflammatory factors, leading to a significant increase in IL-6 and IL-1β secretion levels (**Figure 2A**). Further indirect immunofluorescence (**Figure 2B**) and Western blot (**Figure 2C**) also showed similar results. On this basis, we detected the activation level of NF-κB. The findings indicated a significant increase in NF-κB activation levels following TBBPS/TCBPA treatment (**Figure 2D**).

TBBPS/TCBPA induce NF-κB activation via the cGAS-STING pathway

Previous studies have shown that TBBPS/TCBPA can activate NF-κB. Therefore, a critical question is how TBBPS/TCBPA activate NF-κB in cervical cancer cells. Previous work has shown that TBBPS/TCBPA can promote the production of dsDNA, which can induce an innate immune response. Building on these findings, we first analyzed the expression of inflammasomes. The findings indicated that TBBPS/TCBPA did not significantly elevate AIM2 and NLRP1 expression levels (**Figure 3A**). Consequently, we also examined the cGAS-STING signaling pathway, a nucleic acid-sensing pathway. Treatment with TBBPS/TCBPA activated the cGAS-STING/IRF3 signaling pathway (**Figure 3B**). To verify the necessity of cGAS-STING in NF-κB activation, we employed a cGAS inhibitor. The findings indicated notable suppression of NF-κB activation (**Figure 3C**). Simultaneously, IL-6 and IL-1β levels decreased significantly (**Figure 3D**). Moreover, cell proliferation was significantly attenuated (**Figure 3E**).

TBBPS/TCBPA activate the cGAS-STING signaling pathway by triggering the release of mitochondrial-derived ds-DNA

The study investigates whether TBBPS/TCBPA, functioning as a DAMP molecule, triggers the cGAS-STING signaling pathway by facilitating dsDNA release. Therefore, we detected the levels of dsDNA. Treatment with TBBPS/TCBPA led to a significant increase in dsDNA levels (**Figure 4A**). Next, do these dsDNA molecules come from mitochondria or the nucleus? Theoretically, both sources are possible. Therefore, we inhibited mitochondrial DNA replication using ethidium bromide (EtBR) (**Figure 4B**). The findings indicated a significant reduction in cytoplasmic dsDNA levels following TBBPS/TCBPA treatment, suggesting that dsDNA primarily originates from mitochondria rather than the nucleus. Further colocalization experiments showed that dsDNA mainly originates from mitochondria (**Figure 4C**). TBBPS/TCBPA induce the release of dsDNA from mitochondria into the cytoplasm, activating cGAS-STING and subsequently the NF-κB signaling pathway.

Impact of TBBPS/TCBPA on cervical epithelial cell activity

CCK8 assays were conducted to evaluate the impact of TBBPS/TCBPA on the proliferation of

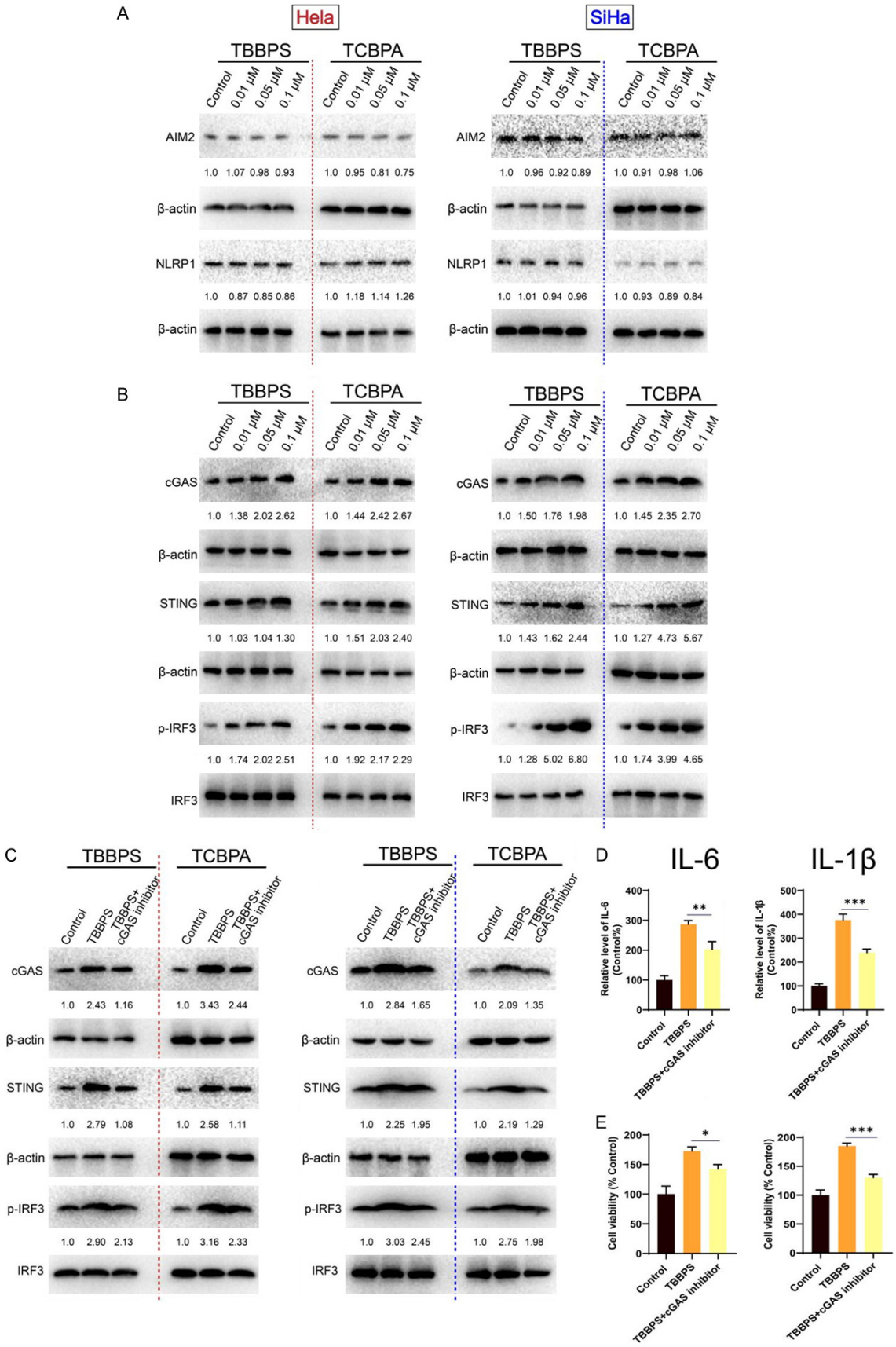
A
Hela
SiHa
TBBPS
TCBPA
Control
0.01 μM
0.05 μM
0.1 μM
AIM2
β-actin
NLRP1
B
cGAS
STING
p-IRF3
IRF3
C
TBBPS+ cGAS inhibitor
D
IL-6
IL-1β
Relative level of IL-6 (Control%)
Relative level of IL-1β (Control%)
E
Cell viability (% Control)
Control
TBBPS
TBBPS+cGAS inhibitor

Figure 3. TBBPS/TCBPA activate NF-κB via the cGAS-STING signaling pathway. A. Evaluation of AIM2 and NLRP1 expression levels following TBBPS/TCBPA treatment. B. TBBPS/TCBPA treatment activated the cGAS-STING/IRF3 signaling pathway. C. NF-κB activation occurred via the cGAS-STING signaling pathway. D. The cGAS inhibitor decreased the expressions of IL-6 and IL-1β. E. The cell-proliferation ability was weakened by using the cGAS inhibitor. A *P*-value less than 0.05 denotes a statistically significant difference.

normal cervical cells (Ect1/E6E7). TBBPS and TCBPA were tested at concentrations ranging from 0.01 to 10 μM (0.01, 0.05, 0.1, 0.5, 1, 5, and 10 μM). The findings indicated that TBBPS/TCBPA suppressed cervical epithelial cell proliferation compared with the control group. In contrast, TBBPS exhibited a stronger inhibitory effect than TCBPA (**Figure 5A**). TBBPS and TCBPA inhibited the proliferation of normal cervical epithelial cells in the EdU experiment (**Figure 5B**). We subsequently assessed the impact of TBBPS/TCBPA on cell-cycle regulatory proteins and proliferation markers. Our data indicated that TBBPS/TCBPA significantly down-regulated the expression levels of Cyclin D1 and Ki67, as shown in **Figure 5C**.

TBBPS/TCBPA trigger pyroptosis via the cGAS-STING-NLRP3 signaling pathway

The above-mentioned experiments showed that TBBPS/TCBPA led to the inhibition of the proliferation of cervical epithelial cells. Through PI and Sytox staining, we found that TBBPS/TCBPA induced the death of cervical cells (**Figure 6A**). This also indicates that this cell-death mode is inflammatory death. To determine the specific form of death, we used inhibitors of pyroptosis, apoptosis, and programmed necrosis. The findings indicated that the cell pyroptosis inhibitor effectively prevented cell death, unlike other inhibitors (**Figure 6B**).

Next, we continued to explore the molecular mechanism by which TBBPS/TCBPA induce cell pyroptosis. Subsequent research demonstrated that TBBPS/TCBPA activated the cGAS-STING pathway (**Figure 6C**). Research indicates that cGAS-STING can trigger NLRP3-mediated cell pyroptosis. Therefore, we further evaluated the expression levels of NLRP3, ASC, GSDMD, and Caspase 1. The findings indicated an overall increase in their expression levels (**Figure 6D**). A cGAS-STING inhibitor was employed to determine if TCBPA/TBBPA activate NLRP3 through the cGAS-STING pathway. The findings indicated that the cGAS-STING inhibitor successfully suppressed NLRP3-mediated pyroptosis (**Figure 6E**). The data suggest that TBBPS/TCBPA trigger cell pyroptosis via the cGAS-STING-NLRP3 signaling pathway.

Discussion

TBBPS and TCBPA have been increasingly used as alternatives to TBBPA in industrial applications in recent years. Currently, due to TBBPA's potential endocrine-disrupting effects and neurotoxicity, it has been restricted for use in many countries. Consequently, TBBPS and TCBPA, as TBBPA's alternatives, have gradually become the main components of flame retardants and plastic additives. However, with the widespread application of TBBPS and TCBPA, environmental contamination issues have gradually emerged. Research indicates that TBBPS and TCBPA are commonly found in diverse environmental media, including water, soil, and indoor dust, as well as biological samples such as human blood and milk [17]. The environmental persistence and bioaccumulation of TBBPS and TCBPA facilitate their entry into the human body via the food chain, potentially threatening human health. Therefore, comprehensively evaluating the toxicological properties of TBBPS and TCBPA, especially their effects on human health, has become an important research direction in the field of environmental health.

In this study, we primarily assessed the effects of TBBPS and TCBPA on cervical cancer. Additionally, we evaluated their effects on cervical cells and reported significant findings. The study revealed that TBBPS and TCBPA significantly enhance cervical cancer cell proliferation and upregulate the expression of various proliferation-related molecules. This finding is consistent with recent studies on the cancer-promoting effects of brominated flame retardants. TBBPA has been shown to enhance the invasion and migration of endometrial cancer cells, potentially through mechanisms involving EMT [18]. The current work shows that TBBPS and TCBPA can induce cervical cancer cells to secrete inflammatory factors, thereby promoting cell proliferation. This phenomenon aligns with the role of inflammatory factors in the cer-

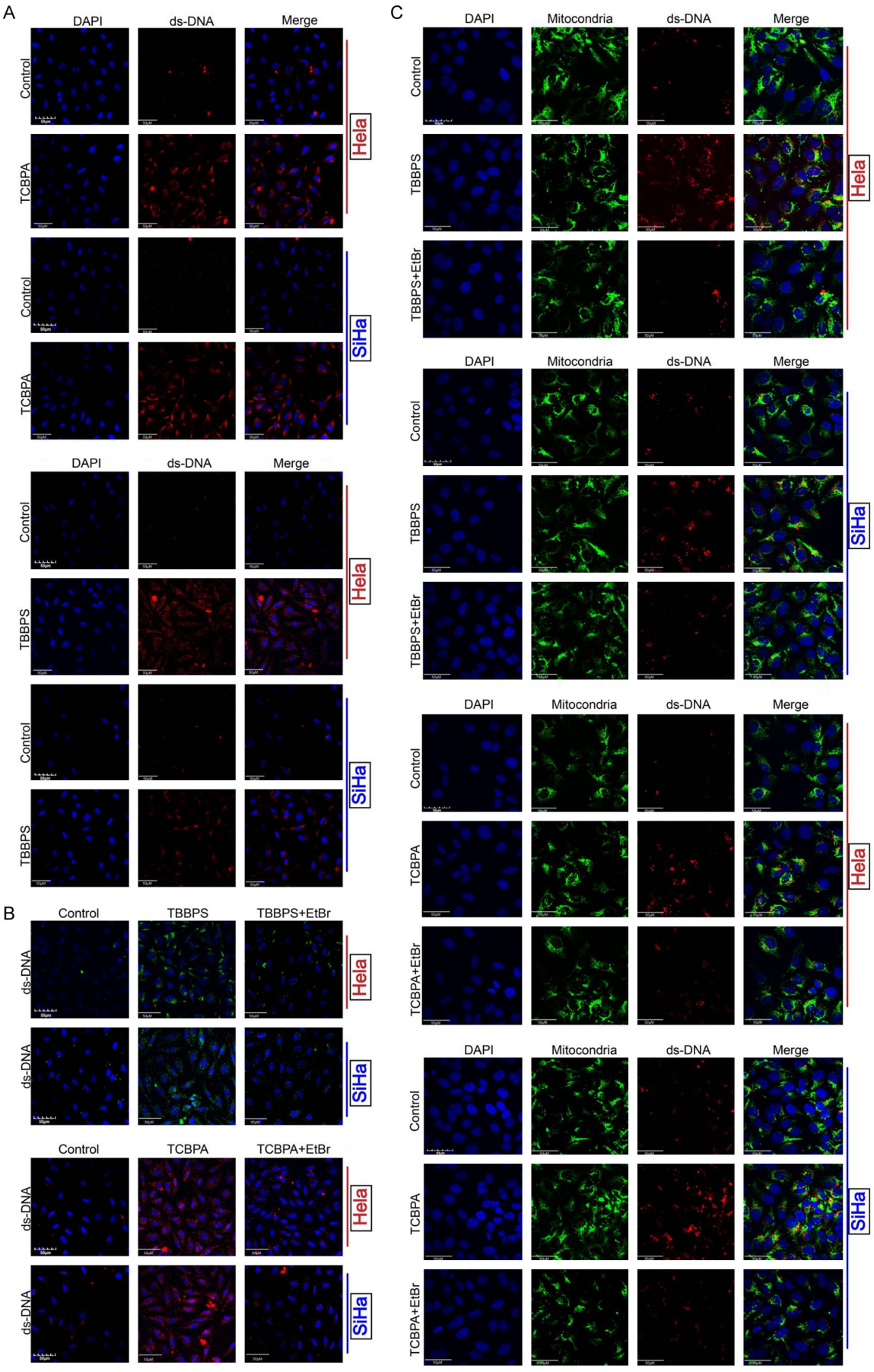
A
DAPI
ds-DNA
Merge
Control
TCBPA
Hela
SiHa
TBBPS
B
Control
TBBPS
TBBPS+EtBr
TCBPA
TCBPA+EtBr
ds-DNA
C
Mitocondria
TBBPS+EtBr
TCBPA+EtBr

Figure 4. TBBPS/TCBPA activate cGAS-STING by inducing the release of mitochondrial-derived ds-DNA. A. TBBPS/TCBPA treatment significantly elevated ds-DNA levels. B. EtBR removes mitochondrial DNA. C. Co-localization analysis of ds-DNA and mitochondria. A *p*-value less than 0.05 signifies a statistically significant difference.

vical cancer microenvironment. Consequently, TBBPS and TCBPA might promote cervical cancer progression by modulating the inflammatory response and tumor immune microenvironment.

This study discovered that, unlike their enhancing effects on cervical cancer cells, TBBPS and TCBPA inhibit the proliferation of normal cervical epithelial cells. The dual effect may be associated with the DNA-damaging effects of TBBPS and TCBPA on cells. Our study demonstrates that TBBPS and TCBPA treatments promote dsDNA release, activating the cGAS-STING pathway and subsequently inhibiting cell proliferation. This is similar to the toxic mechanism of bisphenol-type compounds (BPs). BPA has been shown to suppress normal human cell proliferation and trigger apoptosis through DNA damage and oxidative stress. The inhibitory effects of TBBPS and TCBPA on normal cells may be linked to their endocrine-disrupting effects. BPs can disrupt endogenous hormone synthesis and metabolism, thereby impacting cellular functions [19]. Thus, TBBPS and TCBPA may damage normal cervical cells through various mechanisms.

This study demonstrates that TBBPS and TCBPA can exert dual effects on cervical cancer and normal cervical cells through activation of the cGAS-STING signaling pathway. The cGAS-STING pathway is crucial in immune responses and DNA repair [20, 21]. Our work found that treatment with TBBPS and TCBPA induced the release of dsDNA, which in turn activated the cGAS-STING pathway, ultimately resulting in the inhibition of cell proliferation in the normal cervical cell model. However, this is also understandable due to their effects on inflammatory factors. In the cervical cancer cell model, TBBPS and TCBPA promote cell proliferation by modulating inflammatory factor expression, which in turn influences tumor proliferation and metastasis.

This study shows that TBBPS and TCBPA can induce oxidative stress by disrupting cellular metabolic homeostasis. In liver cells, exposure to TBBPS and TCBPA can significantly increase markers associated with oxidative stress [22]. Qiao et al.'s research shows that TCBPA can induce oxidative stress in vascular endothelial cells and disrupt the cellular redox balance system [23]. Future research should investigate the molecular mechanisms of TBBPS and TCBPA, as well as their toxicological differences compared with TBBPA.

While HPV is the primary driver of cervical cancer, growing evidence suggests that environmental chemicals may act as cofactors in HPV-mediated carcinogenesis. Some environmental factors are known to disrupt immune surveillance against HPV [24]. Here, we hypothesize that TCBPA/TBBPS and HPV may have a synergistic effect on the initiation and progression of cervical cancer. Notably, this is just a hypothesis, and a series of experiments will be needed to confirm it.

Additionally, we found no evidence that TCBPA/TBBPS induce pyroptosis in cervical cancer cells. We hypothesize two possible explanations: First, cervical cancer cells may express very low basal levels of pyroptosis-executing molecules such as Caspase1 (4) and GSDMD compared to normal cervical epithelial cells; Second, TCBPA/TBBPS might inhibit pyroptotic responses in cervical cancer cells through unknown pathways.

Conclusion

The novelty of this study lies in identifying the dual impact of TBBPS and TCBPA on both cervical cancer and normal cervical cells, elucidating the molecular mechanism by which they regulate cell proliferation via the cGAS-STING signaling pathway. This discovery not only provides a scientific basis for assessing the potential health risks of exposure to these compounds but also offers insights for developing intervention strategies against their toxicity. This study presents a novel perspective on the link between environmental pollutants and cancer progression. Therefore, investigating the toxicological properties and molecular mechanisms of TBBPS and TCBPA is crucial for protecting human health and environmental

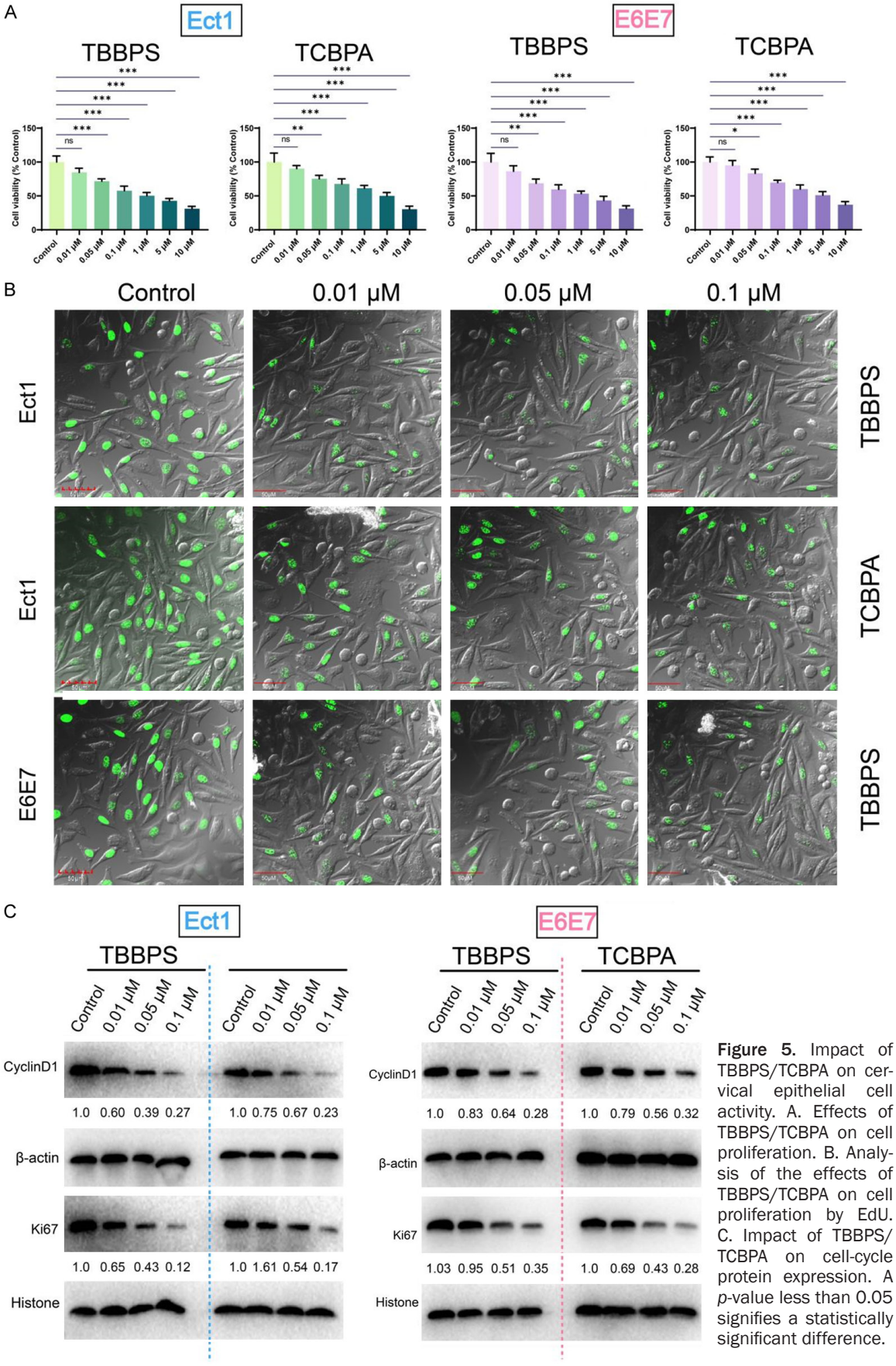

Figure 5. Impact of TBBPS/TCBPA on cervical epithelial cell activity. A. Effects of TBBPS/TCBPA on cell proliferation. B. Analysis of the effects of TBBPS/TCBPA on cell proliferation by EdU. C. Impact of TBBPS/TCBPA on cell-cycle protein expression. A *p*-value less than 0.05 signifies a statistically significant difference.

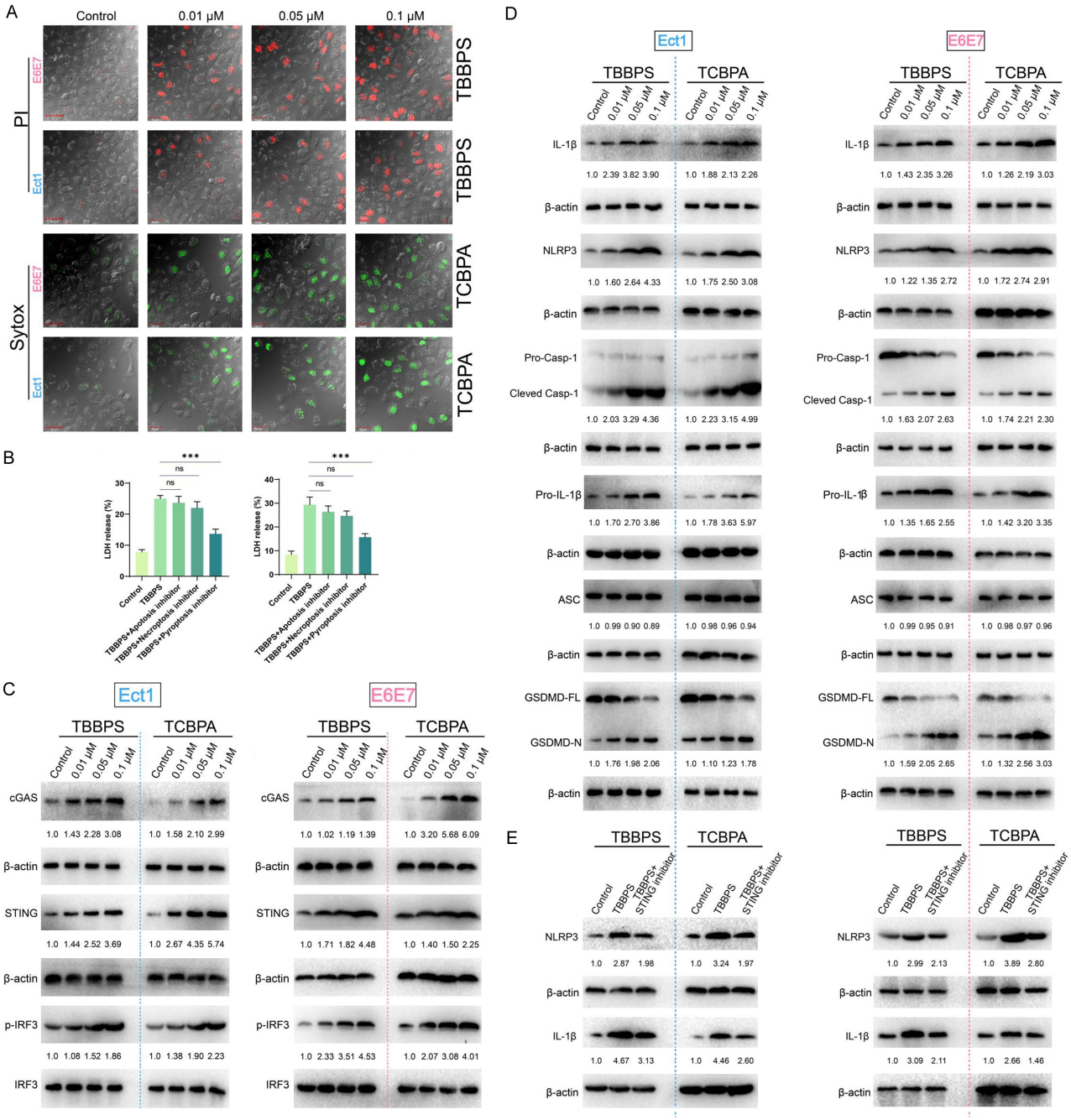
A
Control
0.01 μM
0.05 μM
0.1 μM
PI
Sytox
E6E7
Ect1
TBBPS
TCBPA
B
LDH release (%)
Control
TBBPS
TBBPS+Apotosis inhibitor
TBBPS+Necroptosis inhibitor
TBBPS+Pyroptosis inhibitor
C
Ect1
E6E7
cGAS
STING
p-IRF3
IRF3
β-actin
D
IL-1β
NLRP3
Pro-Casp-1
Cleved Casp-1
Pro-IL-1β
ASC
GSDMD-FL
GSDMD-N
E
TBBPS+ STING inhibitor

Figure 6. TBBPS/TCBPA triggers pyroptosis via the cGAS-STING-NLRP3 signaling pathway. A. TBBPS/TCBPA induced cell death through PI and Sytox staining. B. Inhibition of cell pyroptosis blocked cell pyroptosis. C. TBBPS/TCBPA activated the cGAS-STING signaling pathway in the epithelial cell model. D. Impact of TBBPS/TCBPA on NLRP3, ASC, GSDMD, and Caspase 1 expression levels. E. Pyroptosis mediated by NLRP3 occurs via the cGAS-STING signaling pathway. A *p*-value less than 0.05 signifies a statistically significant difference.

safety. Building on previous findings, this study further delineates the dual impact of TBBPS and TCBPA on cervical cancer and normal cervical cells, highlighting their regulation of cell proliferation via the cGAS-STING signaling pathway. These findings provide critical evidence for assessing the potential health risks of exposure to TBBPS and TCBPA and establish a theoretical basis for developing intervention strategies against their toxicity.

Disclosure of conflict of interest

None.

Abbreviations

TBBPA, tetrabromobisphenol A; BFRs, Brominated flame retardants; PVDF, polyvinylidene fluoride.

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