BMC Pharmacology and Toxicology

RESEARCH

Open Access

# Prodelphinidin B-2,3,3″-O-gallate via chemical oxidation of epigallocatechin-3-gallate shows high efficacy inhibiting triple-negative breast cancer cells

Jing Wang[1,2†], Yuna Wang[1†], Shuanggou Zhang[1,3†], Hongtao Hu[1,3], Ruohan Zhang[1,3], Chengting Zi[1,3*], Jun Sheng[1*] and Peiyuan Sun[1,2*]

## Abstract

**Background** Triple-negative breast cancer is a clinically aggressive malignancy with poorer outcomes versus other subtypes of breast cancer. Numerous reports have discussed the use of epigallocatechin-3-gallate (EGCG) against various types of cancer. However, the effectiveness of EGCG is limited by its high oxidation and instability. The Notch pathway is critical in breast cancer development and prognosis, and its inhibition is a potential treatment strategy.

**Results** In this study, we investigated the effects of prodelphinidin B-2,3,3″-O-gallate (named PB2,3,3″/OG or compound **2**) via chemical oxidation of EGCG on cell viability and the Notch1 signaling pathway in breast cancer cells. We found that compound **2** showed significant cytotoxicity against triple-negative breast cancer cells, with the half maximal inhibitory concentration ($IC_{50}$) values ranging 20–50 μM. In MDA-MB453 cells, compound **2** inhibited proliferation, clone formation, and the expression of proteins involved in the Notch1 signaling pathway. Furthermore, compound **2** induced cell cycle arrest and apoptosis. Consistent with the results of in-vitro experiments, treatment with compound **2** significantly reduced tumor growth. Mechanistically, compound **2** directly bound to Notch1 with high binding affinity (dissociation constant: $K_D = 4.616 \times 10^{-6}$ M).

**Conclusion** Our finding suggested that compound **2** may be a promising agent for the development of novel anti-cancer therapy options.

**Keywords** Triple-negative breast cancer, Prodelphinidin B-2,3,3''-O-gallate, Cell viability, Apoptosis, Notch1

†Jing Wang, Yuna Wang and Shuanggou Zhang contributed equally to this work.

*Correspondence:
Chengting Zi
zichengting@126.com
Jun Sheng
shengj@ynau.edu.cn
Peiyuan Sun
sunxingjia8888@126.com
[1]Key Laboratory of Pu-er Tea Science, Ministry of Education, Yunnan Agricultural University, Kunming 650201, China
[2]College of Science, Yunnan Agricultural University, Kunming 650201, China
[3]College of Food Science and Technology, Yunnan Agricultural University, Kunming 650201, China

## Introduction

Breast cancer is common among women worldwide [1]. Triple-negative breast cancer (TNBC) accounts for 15–20% of breast cancer cases and is highly aggressive [2, 3]. Owing to the poor expression of estrogen receptor (ER), progesterone receptor, and human epidermal growth factor receptor 2 (HER2), TNBC fails to respond to therapies targeting these protein receptors [4]. Currently, chemotherapy is the main treatment for patients with TNBC. Although the combination of anthracycline and taxane is an effective therapeutic option, patients with TNBC continue to exhibit poor overall survival. Therefore, novel treatment strategies are urgently required to improve the prognosis and survival of patients with TNBC [5–7].

Notch signaling plays a significant role in cell maintenance, differentiation, proliferation, and apoptosis. More recently, Notch signaling has been implicated in human breast cancers and is involved in the development of breast cancer. Studies have shown that high expression of Notch1 was associated with poor survival in patients with breast cancer [8, 9]. It has been reported that green tea and its most abundant constituent catechin, i.e., epigallocatechin-3-gallate (EGCG), exert beneficial effects on different diseases, including TNBC [10, 11]. Moreover, it has been shown that EGCG can directly bind with Notch1. However, EGCG is highly oxidizable and unstable, limiting its efficacy [2, 12]. Previous research studies demonstrated that EGCG derivatives were more stable than EGCG, and exhibited antitumor activity [13]. Therefore, we hypothesized that EGCG oxide (compound **2**) may exhibit anticancer activity and inhibit the Notch1 sig-naling pathway in breast cancer cells. The objective of this study was to examine the antiproliferative activity of EGCG oxide (prodelphinidin B-2,3,3”-O-gallate [PB2,3,3”/OG]) in TNBC cells.

## Materials and methods

### Materials

All cell lines were purchased from the American Type Culture Collection (Ma-nassas, VA, USA) and cultured in Ham's F12 medium (Biological Industries, Cromwell, CT, USA). Antibodies against poly (ADP-ribose) polymerase 1 (PARP1), cleaved-PARP1 and Ki67 were obtained from Abcam (Cambridge, MA, USA). Antibodies against cyclin E and cyclin D1 were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Antibodies against caspase 3, cleaved-caspase 3, caspase 9, cleaved-caspase 9, cleaved-Notch1 (Val1744), Notch1 (C37C7), cyclin dependent kinase 2 (CDK2), cyclin dependent kinase 4 (CDK4) and glyceralde-hyde-3-phosphate dehydrogenase (GAPDH) were obtained from Cell Signaling Technology (Beverly, MA, USA). The 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2 H-tetrazolium inner salt (MTS) was purchased from Promega (Madison, WI, USA). EGCG was obtained from Chengdu Purifa Technology Development Co., Ltd. (Chengdu, Sichuan, China). Dimethyl sulfoxide (DMSO) was obtained from Amresco (Houston, TX, USA).

### Test compound

PB2,3,3”/OG (or compound **2**) was isolated through the chemical oxidation of EGCG. It was temporarily refered as compound **2** for subsequent experiments. The agent was isolated as a yellow amorphous powder. The characteristic data of compound **2** were reported in the literature [13]. Compound **2** was dissolved in DMSO at a concentration of 200 mM and stored at -20 ℃ until use. For the experiments, the final concentrations of the test compound were prepared by diluting the stock with Ham's F12 medium.

### Cell viability assay

The cell viability was examined using MTS assays. MDA-MB231/453/468 and MCF7 cells were seeded in 96-well plates. After 24 h, the cells were treated with EGCG or compound **2** for another 24 h. Next, the cells were fixed with 20 µL of MTS solution for 3 h, and then the 96-well plates were shaken at room temperature for 10 min. Finally, the values of absorbance in each well were measured using a Flex Station 3 Multi-Mode Microplate Reader (Molecular Devices, San Jose, CA, USA) at 490 nm.

### Colony formation assay

The method of colony formation assay was referred to our previous experimental methods [14]. MDA-MB453 cells ($1.5 \times 10^3$ cells/dish) were seeded into 60-mm cell culture dishes and treated with different doses of compound **2** (10, 20, 40 µM). After 24 h, the cells were cultured in medium supplemented with 10% (v/v) fetal bovine serum (Biological Industries) for 14 days, and the medium was replaced every 2 days. Subsequently, the cell colonies were stained with 0.01% crystal violet solution for 30 min and dissolved with 10% glacial acetic acid. Finally, optical densities at 560 nm were measured using a Flex Station 3 Multi-Mode Microplate Reader (Molecular Devices).

### Cell cycle analysis

MDA-MB453 cells were maintained in six-well plates ($5.0 \times 10^5$ cells/well). After 24 h, the cells were treated with compound **2** for 24 h. Next, the cells were harvested and washed with ice-cold phosphate-buffered saline, fixed in 70% ethanol at 4 ℃ for 12 h, washed twice using cold phosphate-buffered saline, and stained in buffer containing 10 µg/mL of propidium iodide (PI) and RNAse for 30 min in the dark. Cell cycle analysis was performed

through a BD FACSCalibur flow cytometer (BD Biosciences, San Jose, CA, USA).

#### Cell apoptosis assay

MDA-MB453 cells were cultured in six-well plates ($5.0 \times 10^5$ cells/well) and then treated with various doses of compound **2** (10, 20, 40 μM) for 24 h. The cells were subsequently harvested and incubated with Annexin V and propidium iodide (PI) for 15 min in the dark. Finally, the cells were detected using a BD FACSJazz flow cytometer (BD Biosciences).

#### Western blotting analysis

MDA-MB453 cells were treated with compound **2** in cell culture dishes and collected in lysis buffer. The proteins in the samples were separated using sodium dodecyl sulfate-polyacrylamide gel and transferred to polyvinylidene difluoride membranes (EMD Millipore Corporation, Merck Life Sciences, KGaA, Darmstadt, Germany). After blocking with 5% non-fat milk, the membranes were incubated with specific antibodies overnight at 4 ℃. Next, the membranes were incubated with secondary antibodies at room temperature for 1 h. Proteins were visualized with the FluorChem E System (ProteinSimple, Santa Clara, CA, USA).

#### Tumorigenicity assays

Experimental research on nude mice complies with Yunnan Agricultural University guidelines. The animal experiment has been approved by ethics committee. Six-week-old male nude mice were purchased from the Cavens Lab Animal (Changzhou, China). The mice were randomly assigned into two groups, namely control and compound **2** ($n = 6$). MDA-MB-453 cells ($5 \times 10^6$ cells) were subcutaneously injected into the left flanks of nude mice. Next, the mice were treated with saline solution or compound **2** (20 mg/kg) through intraperitoneal daily injection. Tumor volumes were measured with calipers and calculated according to the following formula: Volume = (Length × Width$^2$) / 2.

#### Surface plasmon resonance (SPR) assay

The SPR assays were performed using a Biacore S200 instrument (GE Healthcare) at 25 ℃. Notch1 (30 μg/mL in 10 mM sodium acetate, pH 4.0) was immobilized on the flow cell of the Series S CM5 Sensor Chip using an amine coupling kit (GE Healthcare). The analyte, compound **2** (0.625, 1.25, 2.5, 5, 10 μM), was injected and passed over the immobilized Notch1 sensor surface. The flow rate was 30 mL/min, while the binding time and the dissociation time were 90 s each. Kinetics analyses were performed using the Biacore S200 Evaluation Software (version 1.1, GE Healthcare).

#### Molecular docking analysis

The three-dimensional structure of the Notch1 (ID: 3ETO) was obtained from the Protein Data Bank (http://www.rcsb.org/pdb/). DiscoveryStudio 2016 software was used for the preparation of ligand and receptor and docking, according to the literature [15, 16]. Autodock Vina 1.1.2 software was used to perform the binding analysis. In the docking calculation, the confirmation with the lowest binding energy was finally selected to further analyze the receptor-ligand interactions. Pymol 1.5 software was utilized to visualize the three-dimensional model of compound **2** and Notch1, while the two-dimensional model was visualized by DiscoveryStudio 2016 software.

#### Statistical analysis

All data were presented as the mean ± standard deviation (SD) and analyzed using the GraphPad Prism 5.03 software. Differences were examined using Student's t-test or one-way analysis of variance, and p-values < 0.05 denoted statistically significant differences.

## Results

#### Preparation of PB2,3,3″/OG (compound 2)

The chemical oxidation of tea catechins under various oxidative conditions produces theaflavins and theasinensins [17–19]. Previously, we reported that various EGCG oxides are produced by the oxidative condition of K3[Fe(CN)6]/NaHCO3 [13]. In this study, PB2,3,3″/OG was prepared using a similar method previously reported in the literature (as shown in Figure S1).

#### Compound 2 inhibited the proliferation of breast cancer cells in vitro and in vivo

Chemical structure of compound **2** is shown in Fig. 1. To determine whether compound **2** inhibits the proliferation of breast cancer cells, we treated TNBC cell lines (MDA-MB231/453/468) and an ERα-positive breast cancer cell line (MCF7) with EGCG or compound **2** for 24 h. Compared with EGCG (40 μM), compound **2** (40 μM) showed high cytotoxicity (Fig. 2A). Subsequently, we treated these breast cancer cell lines with increasing doses of compound **2**. Compound **2** markedly inhibited the proliferation of breast cancer cells in a dose-dependent manner (Fig. 2B). In MDA-MB231/453/468 and MCF7 cells, the half maximal inhibitory concentration ($IC_{50}$) values of compound **2** were 33.875 ± 1.764 μM, 28.006 ± 0.983 μM, 49.0093 ± 2.660 μM, and 41.97 ± 1.787 μM, respectively. The $IC_{50}$ values in these cell lines ranged 20–50 μM, indicating that compound **2** may not exhibit cell type specificity in these breast cancer cell lines.

We determined the effects of compound **2** on tumor growth using an established xenograft generated by subcutaneous dorsal implantations of MDA-MB453 cells into nude mice. Compound **2** (20 mg/kg) did not reduce

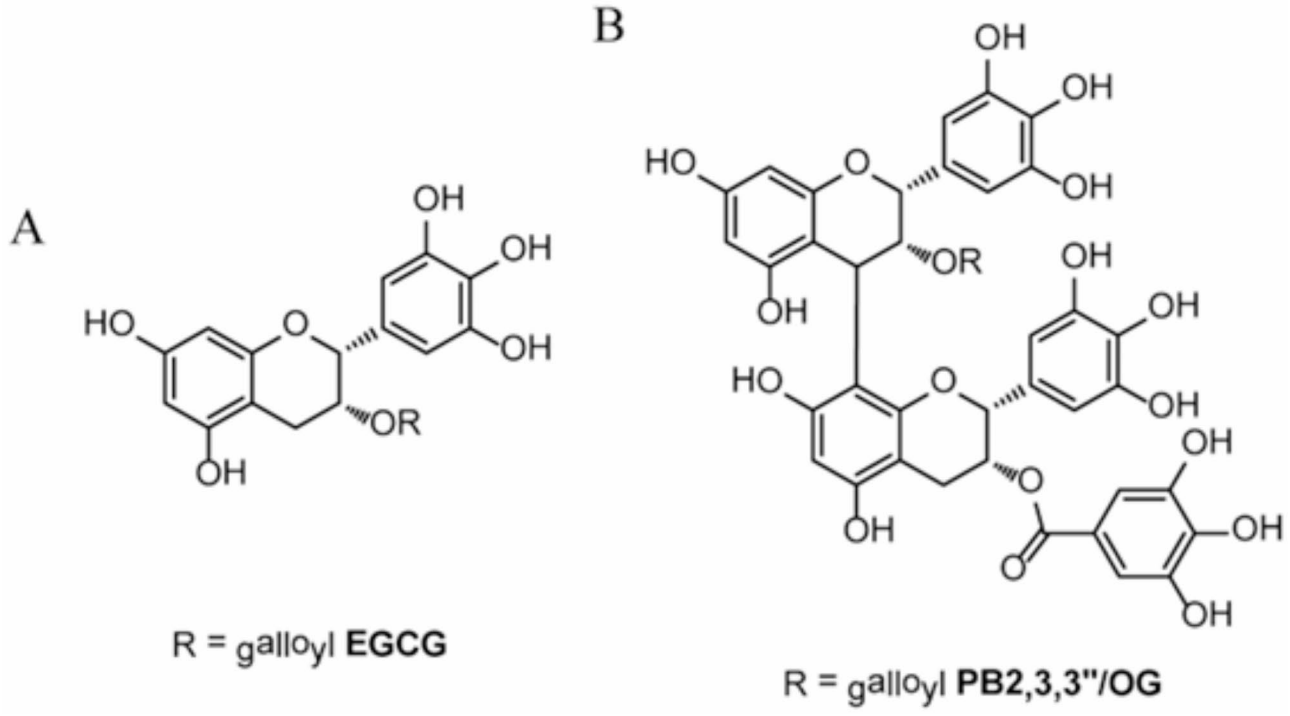


**Fig. 1** Chemical structures of EGCG (**A**) and PB2,3,3″/OG (**B**)

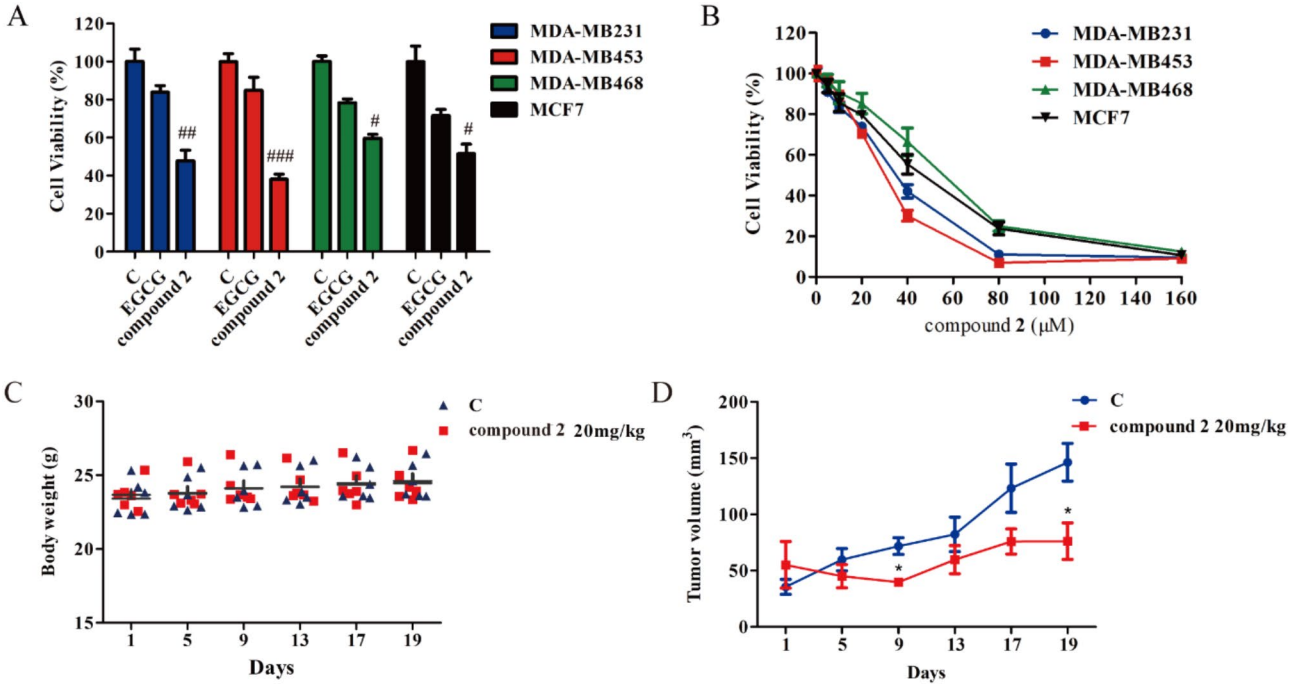


**Fig. 2** Effects of EGCG and its oxide (compound **2**) on the proliferation of breast cancer cells in vitro and in vivo. (**A**) Three TNBC cell lines (MDA-MB231/453/468) and an ERα-positive breast cancer cell line (MCF7) were treated with EGCG (40 μM) or compound **2** (40 μM) for 24 h. Cell proliferation was measured using the MTS assay. Results are expressed as the mean ± SD ($n = 3$). #$p < 0.05$, ##$p < 0.01$, ###$p < 0.001$ versus EGCG. (**B**) The cells were treated with compound **2** (5, 10, 20, 40, 80, and 160 μM) for 24 h. All values are expressed as the mean ± SD ($n = 3$). (**C**) Data on the body weight of mice in each group were analyzed. (**D**) Tumor volumes were measured with calipers and analyzed. *$p < 0.05$ versus control

the body weight of mice (Fig. 2C). Consistent with the results of the in vitro experiments, compound **2** (20 mg/kg) inhibited the growth of the MDA-MB453 xenograft tumor compared with control (Fig. 2D).

### Compound 2 inhibited clone formation of MDA-MB453 cells

We further performed a colony formation assay to determine the anti-proliferative potential of compound **2**. As the concentration of compound **2** increased, the number of cells gradually decreased and showed a dose-dependent effect (Fig. 3A and B), indicating that high concentrations of compound **2** can significantly inhibit the proliferation of MDA-MB453 cells. Moreover, we detected the expression levels of proteins associated with cell growth and proliferation by western blotting. Following treatment with compound **2**, the expression of Ki67 decreased (Fig. 3C).

### Compound 2 induced S-phase cell cycle arrest in MDA-MB453 cells

Given that compound **2** inhibited the proliferation of TNBC cells, we investigated whether it could also induce cell cycle redistribution. Hence, we treated MDA-MB453 cells with compound **2** and stained them with PI. Figure 4A and B show that compound **2** significantly increased the percentage of cells in the S phase compared with control. We further examined the expression of several cell cycle proteins using western blotting. The expression levels of cyclin E were increased in MDA-MB453 cells following treatment with compound **2** (Fig. 4C).

### Compound 2 induced apoptosis in MDA-MB453 cells

We sought to investigate whether compound **2** induces apoptosis in MDA-MB453 cells. For this purpose, we treated these cells with compound **2** and measured the degree of apoptosis via Annexin V/PI staining and flow cytometry. Compound **2** (20 μM) drastically increased the proportions of Annexin V-positive apoptotic cells in a dose-dependent manner (Fig. 5A and B). Furthermore, we examined the expression levels of apoptosis-related proteins by western blotting. The expression levels of cleaved-caspase3/9 and cleaved-PARP1 were markedly increased, suggesting that caspase activation may play an important role in compound **2**-induced apoptosis in MDA-MB453 cells (Fig. 5C).

### Compound 2 blocked Notch1 processing in MDA-MB453 cells

Some studies indicated that EGCG regulates Notch signaling in the development of several types of cancer [18, 19]. Thus, we treated MDA-MB453 cells with compound **2** and detected the expression levels of proteins associated with the Notch1 signaling pathway by western blotting. The protein expression levels of cleaved-Notch1 in MDA-MB453 cells were decreased after treatment with compound **2**, while the protein expression levels of Notch1 remained relatively stable (Fig. 6). These data indicated that compound **2** may regulate Notch1 signaling in MDA-MB453 cells.

### Compound 2 directly bound to Notch1 with high affinity

To investigate whether compound **2** directly interacts with Notch1, we evaluated their binding affinity using SPR technology. As shown in Fig. 7A, compound **2**

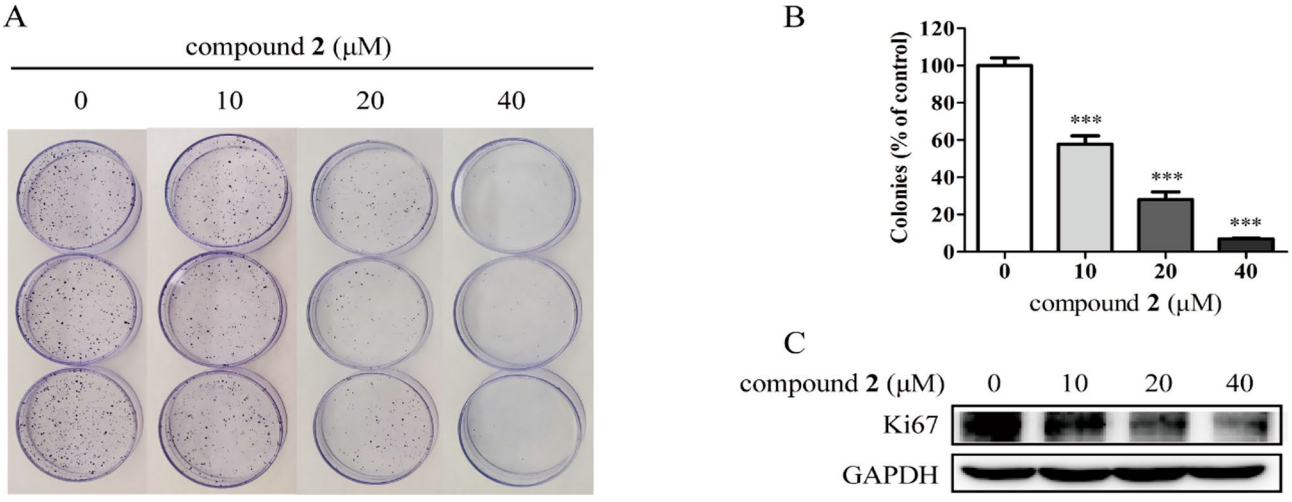


**Fig. 3** Effects of compound **2** on the clone formation of MDA-MB453 cells. (**A**) Cells were treated with compound **2** (10, 20, 40 μM) for 24 h. After 14 days, colony formation was stained with 0.01% crystal violet solution and assessed. (**B**) The cell colonies were dissolved with 10% glacial acetic acid, and the optical densities of the samples were measured at 560 nm. The ratio of clone formation in each group is expressed as percentage. All values are expressed as the mean ± SD ($n = 3$). ***$p < 0.001$ versus control. (**C**) Cells were treated with compound **2** (10, 20, 40 μM). The protein expression of Ki67 was detected by western blotting. GAPDH was used as the loading control

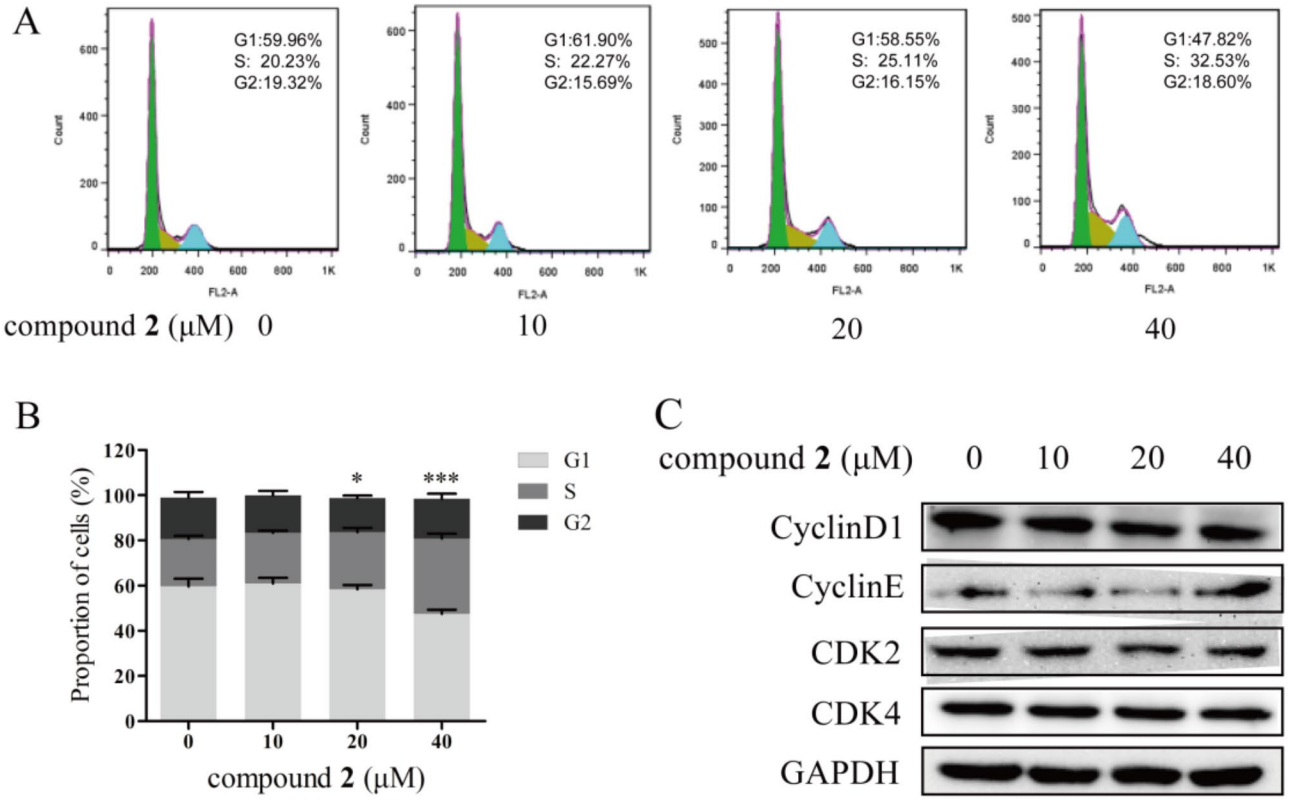


**Fig. 4** Compound **2** induced S-phase cell cycle arrest in MDA-MB453 cells. (**A**) Cells were treated with compound **2** (10, 20, 40 μM) for 24 h. The cell cycle distribution was analyzed using the FlowJo software. (**B**) The quantification of the cell populations in each phase of the cell cycle is presented. All values are expressed as the mean ± SD ($n = 3$). *$p < 0.05$, ***$p < 0.001$ versus control. (**C**) Cells were treated with compound **2** (10, 20, 40 μM), and the expression levels of cell cycle-related proteins were determined through western blotting

directly interacted with Notch1, and the binding affinity was $4.616 \times 10^{-6}$ M. Next, we performed a molecular docking to further investigate the possible binding mode of compound **2** to Notch1. Figure 7B illustrates that compound **2** is docked in the Notch1. Moreover, compound **2** binds to the Notch1 by forming hydrogen bonds with SER-1712, HIS-1602, GLU-1526, PHE-1520, ASP-1521 and GLY-1504 (Fig. 7B-D). The binding energy between compound **2** and the Notch1 was estimated to be -8.6 kcal/mol. These results suggested that compound **2** interacts strongly with Notch1.

## Discussion

Breast cancer remains a major health problem among women worldwide [20]. Although targeted therapies have markedly prolonged the survival of patients with ER-positive tumors, they exhibit limited efficacy against TNBC [4]. Numerous studies indicated that endocrine therapy or ER-directed therapies were ineffective against TNBC, highlighting the requirement for other treatment approaches [21, 22]. Currently, the most effective treatment for TNBC is cytotoxic chemotherapy. Nevertheless, this type of therapy is characterized by high rates of resistance and rapid relapse. Accumulating evidence suggests that resistance to chemotherapy and immune evasion are typically associated with genomic and chromosomal instability in early-stage TNBC [23]. The anticancer activity of EGCG has been widely investigated in vitro and in vivo [9]. However, the potential usefulness of EGCG has been hampered by its high oxidation and instability [12, 24]. In our previous study, an EGCG oxide (compound **2**) was reported. Despite its solubility not being significantly improved, compound **2** showed high stability and activity in reducing cell viability in T-cell acute lymphoblastic leukemia (T-ALL), as well as in suppressing the expression of proteins involved in the Notch1 signaling pathway, compared with EGCG [13]. Therefore, we hypothesized that the EGCG oxide (compound **2**) may also exert similar effects on the viability of breast cancer cells and the Notch1 signaling pathway. In the present study, we evaluated the effects of EGCG oxide (compound **2**) on the viability of TNBC cells in vitro and in vivo, and investigated the possible mechanisms involved in this process.

In this study, we first tested compound **2** on the viability of breast cancer cells, including MDA-MB231/453/468 and MCF7 cells. The results indicated that compound **2** exerted similar anti-proliferative

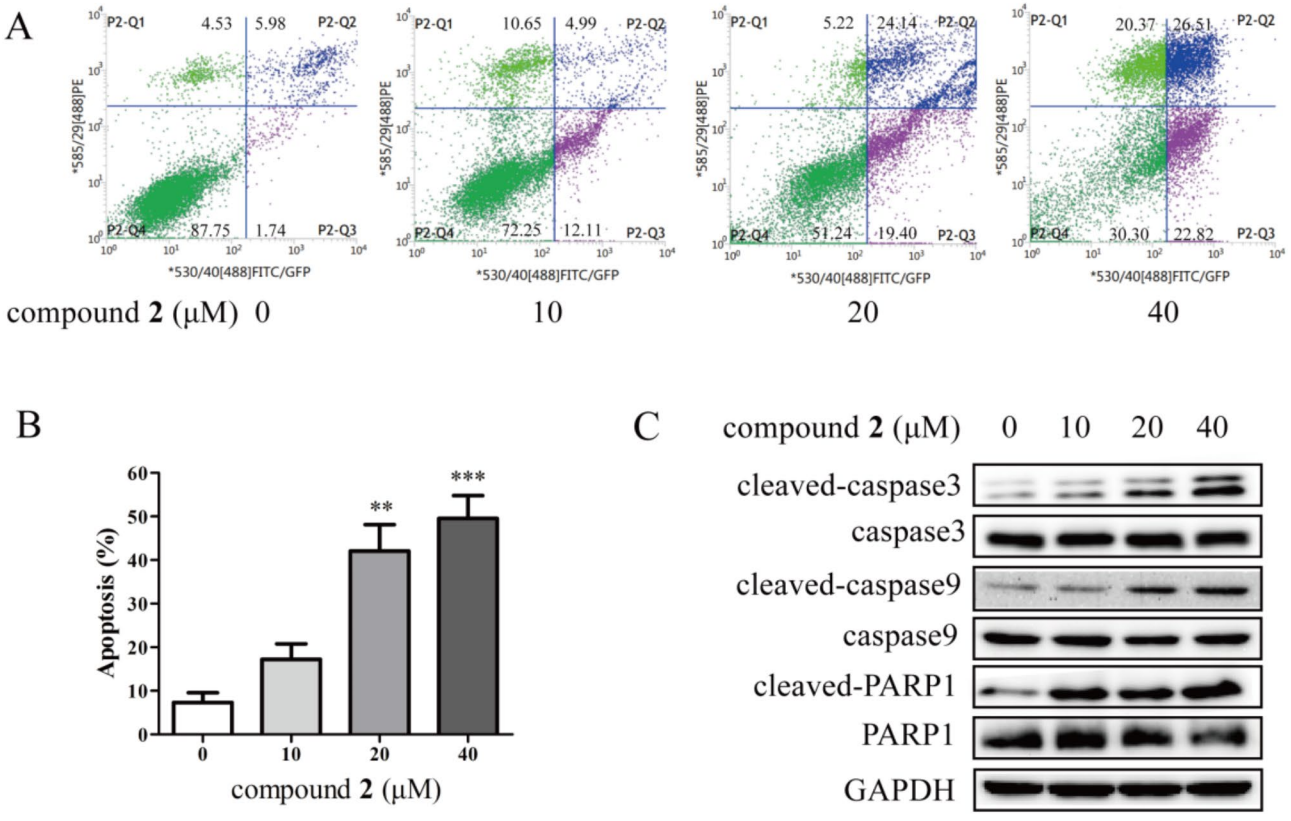


**Fig. 5** Compound **2** induced apoptosis in MDA-MB453 cells. (**A**) Flow cytometry of MDA-MB453 cells after treatment with compound **2** (10, 20, 40 μM) for 24 h. (**B**) The ratio of apoptotic cells in each group is expressed as percentage. All values are expressed as the mean ± SD ($n = 3$). $^{**}p < 0.01$, $^{***}p < 0.001$ versus control. (**C**) Cells were treated with compound **2** (10, 20, 40 μM), and the expression levels of cleaved-caspase3/9, caspase3/9, cleaved-PARP1, and PARP1 were detected by western blotting. GAPDH was used as the loading control

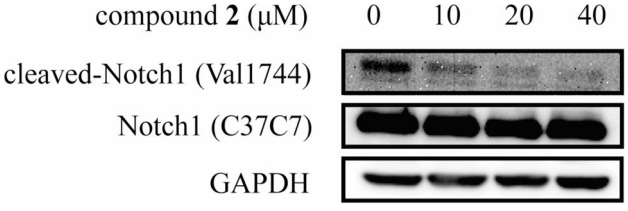


**Fig. 6** Effect of compound **2** on Notch1 processing in MDA-MB453 cells. Cells were treated with compound **2** (10, 20, 40 μM) for 24 h. The protein expression levels of Notch1 and cleaved-Notch1 were detected by western blotting

effects on these cells, with $IC_{50}$ values ranging from 20 to 50 μM. Given that compound **2** exhibited the lowest $IC_{50}$ value in MDA-MB453 cells, we selected MDA-MB453 cells as the study model for subsequent assays. It has been reported that the anti-proliferative activity of EGCG led to apoptosis [25, 26]. Similarly, we found that compound **2** markedly inhibited the proliferation and induced apoptosis of MDA-MB453 cells. These effects may be associated with a reduction in the expression of the proliferation marker Ki67 and an increased activation of caspase3/9 and PARP1 induced by compound **2**. Meanwhile, some studies have also reported that the regulation of Notch1 signaling is involved in cell apoptosis in a caspase-dependent manner [27, 28]. Moreover, we found that compound **2** may induce cell cycle redistribution in MDA-MB453 cells, leading to cell cycle arrest in the S phase. Based on these findings, compound **2** exhibits antitumor activity against TNBC through anti-proliferative effects, pro-apoptosis effects and cell cycle redistribution.

Notch signaling regulates diverse cellular processes involved in cell fate, stem cell self-renewal, and tissue differentiation [29]. Accumulating evidence suggests that dysregulation of the Notch pathway is conducive to carcinogenesis and the development of resistance to chemotherapy [30]. Numerous studies, including our previous work, have indicated that EGCG directly binds to Notch and regulates Notch signaling in the development of inflammation and different types of cancer [31–36]. Furthermore, in one of our previous studies, the results suggested that compound **2** suppresses Notch1 signaling in T-ALL, yet the binding mechanism between the agent and Notch1 was not further investigated [13]. In this study, we used SPR technology-based molecular interaction assays and molecular docking analysis to investigate

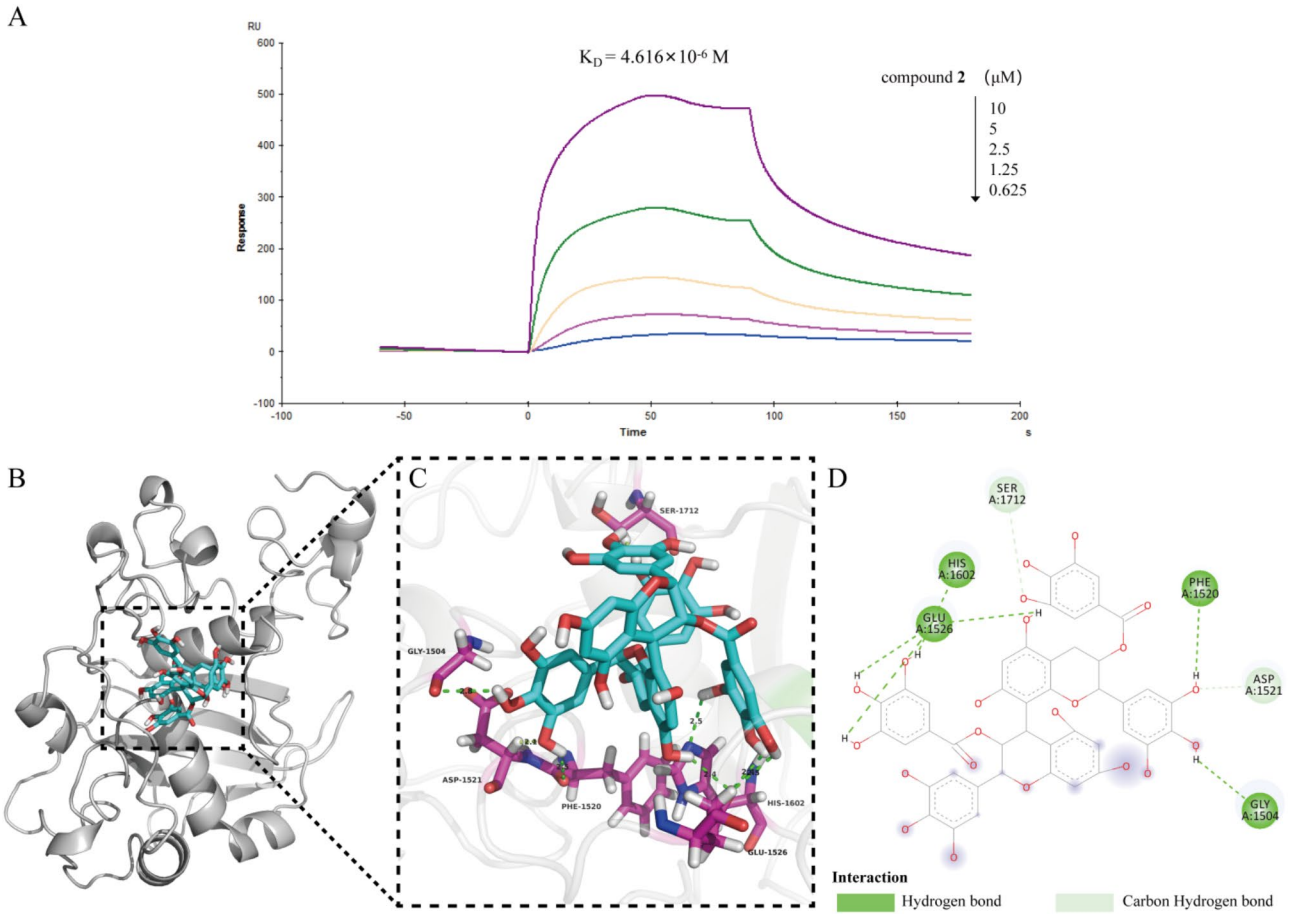


**Fig. 7** Binding mode of compound **2** and Notch1. (**A**) Compound **2** directly interacted with Notch1, with a $K_D$ value of $4.616 \times 10^{-6}$ M. (**B**, **C**) Molecular docking model of compound **2** bound to the Notch1. Ligand and key residues are shown as sticks, and interactions are shown as dashed lines. (**D**) Two-dimensional diagram of compound **2**-Notch1 interactions. $K_D$, dissociation constant

the binding affinity and mode between compound **2** and Notch1. We found that compound **2** strongly interacts with Notch1. These results suggest that compound **2** suppresses Notch1 signaling via direct binding to Notch1, thereby hindering the progression of TNBC. Additionally, in our previous study, EGCG interacted with Notch1 with a $K_D$ value of $2.04 \times 10^{-6}$ M, which is comparable to the $K_D$ value of compound **2** binding to Notch1 ($4.616 \times 10^{-6}$ M) in this study [35]. Therefore, we suspected that the differences in cytotoxic effects between compound **2** and EGCG on MDA-MB453 cells may be related to the enhanced stability of compound **2** compared to EGCG. In the future, more experiments are needed to further validate these findings.

## Conclusions

Collectively, our results demonstrated that EGCG oxide (compound **2**) exhibits antitumor activity against TNBC, including inhibiting the viability of TNBC cells, reducing growth of TNBC xenograft tumors, promoting cell apoptosis and inducing cell cycle arrest. These effects are involved in directly interacting with Notch1 with a high binding affinity. These findings suggest that compound **2** is a candidate for the development of novel treatment strategies against TNBC.

## Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40360-025-00883-6.

Supplementary Material 1

Supplementary Material 2

### Author contributions

J.S., C.Z., J.W. and P.S. designed the study. Y.W., J.W., S.Z. and H.H. performed experiments. Y.W., H.H., R.Z. and S.Z. analyzed the data. P.S., Y.W., S.Z. and J.W. wrote the manuscript. J.S., C.Z. and P.S. reviewed the manuscript. All authors read and approved the final manuscript.

### Funding

This research was funded by the Project of the Yunnan Province Agricultural Basic Research Joint Foundation (202401BD070001-056), the Yunnan Fundamental Research Project (202201AU070177) and the Project of Yunnan International Science and Technology Specialists (202403AK140041).

**Data availability**
Data will be made available on request.

## Declarations

**Ethical approval**
All experiments on nude mice comply with Yunnan Agricultural University guidelines. The experiments carried out in this work have been approved by ethics committee.

**Competing interests**
The authors declare no competing interests.

Received: 31 October 2024 / Accepted: 25 February 2025
Published online: 28 February 2025

**References**

1. Siegel RL, Miller KD, Wagle NS, et al. Cancer statistics, 2023. CA Cancer J Clin. 2023;73(1):17–48.
2. Hammond ME, Hayes DF, Dowsett M, et al. American society of clinical oncology/college of American pathologists guideline recommendations for immunohistochemical testing of Estrogen and progesterone receptors in breast cancer. J Clin Oncol. 2010;28(16):2784–95.
3. Kalimutho M, Parsons K, Mittal D, et al. Targeted therapies for Triple-Negative breast cancer: combating a stubborn disease. Trends Pharmacol Sci. 2015;36(12):822–46.
4. Reinert T, Barrios CH. Optimal management of hormone receptor positive metastatic breast cancer in 2016. Ther Adv Med Oncol. 2015;7(6):304–20.
5. Carey LA, Dees EC, Sawyer L, et al. The triple negative paradox: primary tumor chemosensitivity of breast Cancer subtypes. Clin Cancer Res. 2007;13(8):2329–34.
6. Bianchini G, Balko JM, Mayer IA, et al. Triple-negative breast cancer: challenges and opportunities of a heterogeneous disease. Nat Rev Clin Oncol. 2016;13(11):674–90.
7. Lee A, Djamgoz MBA. Triple negative breast cancer: emerging therapeutic modalities and novel combination therapies. Cancer Treat Rev. 2018;62:110–22.
8. Shao S, Zhao X, Zhang X, et al. Notch1 signaling regulates the epithelial-mesenchymal transition and invasion of breast cancer in a Slug-dependent manner. Mol Cancer. 2015;14(1):28.
9. Negri A, Naponelli V, Rizzi F, et al. Molecular targets of Epigallocatechin-Gallate (EGCG): A special focus on signal transduction and Cancer. Nutrients. 2018;10(12):1936.
10. Bimonte S, Cascella M, Barbieri A, et al. Current shreds of evidence on the anticancer role of EGCG in triple negative breast cancer: an update of the current state of knowledge. Infect Agent Cancer. 2020;15:1–6.
11. Wei R, Mao L, Xu P, et al. Suppressing glucose metabolism with epigallocatechin-3-gallate (EGCG) reduces breast cancer cell growth in preclinical models. Food Funct. 2018;9(11):5682–96.
12. Kitao S, Matsudo T, Saitoh M, et al. Enzymatic syntheses of two stable (-)-epigallocatechin Gallateglucosides by sucrose phosphorylase. Biosci Biotechnol Biochem. 1995;59:2167–9.
13. Wang YN, Wang J, Yang HN, et al. The oxidation of (-)-epigallocatechin-3-gallate inhibits T-Cell acute lymphoblastic leukemia cell line HPB-ALL via the regulation of Notch1 expression. RSC Adv. 2020;10:1679–84.
14. Sun PY, Zhang SG, Qu YN, et al. Coccinic acid exhibits anti-tumor efficacy against NSCLC harboring EGFR L858R/T790M mutation via the EGFR/STAT3 pathway. Bioorg Chem. 2025;154:108038.
15. Wang J, Wang Y, Zhang S, et al. Inhibitory effect of 1,4,5,6-tetrahydroxy-7,8-diprenylxanthone against NSCLC with L858R/T790M/C797S mutant EGFR. Sci Rep. 2024;14(1):26549.
16. Tuo Y, Lu X, Tao F, et al. The potential mechanisms of catechins in tea for anti-Hypertension: an integration of network pharmacology, molecular docking, and molecular dynamics simulation. Foods. 2024;13(17):2685.
17. Granja A, Frias I, Neves AR, et al. Therapeutic potential of Epigallocatechin gallate nanodelivery systems. BioMed Res Int. 2017;2017:1–15.
18. Takino Y, Imagawa H, Aoki Y, et al. Studies on the mechanism of the oxidation of tea leaf catechins. Agric Biol Chem. 1963;27:562–8.
19. Shii T, Miyamoto M, Matsuo Y, et al. Biomimetic one-pot Preparation of a black tea polyphenol theasinensin A from Epigallocatechin gallate by treatment with copper(II) chloride and ascorbic acid. Chem Pharm Bull. 2011;59(9):1183–5.
20. Gao Y, Rankin GO, Tu YY, et al. Theaflavin-3, 3'-digallate decreases human ovarian carcinoma OVCAR-3 cell-induced angiogenesis via Akt and Notch-1 pathways, not via MAPK pathways. Int J Oncol. 2016;48(1):281–92.
21. Jin HY, Gong W, Zhang CX, et al. Epigallocatechin gallate inhibits the proliferation of colorectal cancer cells by regulating Notch signaling. Onco Targets Ther. 2013;6:145–53.
22. Wang Q, Gun M, Hong XY. Induced Tamoxifen resistance is mediated by increased methylation of E-Cadherin in Estrogen Receptor-Expressing breast Cancer cells. Sci Rep. 2019;9(1):14140.
23. Al-Mahmood S, Sapiezynski J, Garbuzenko OB, et al. Metastatic and triple-negative breast cancer: challenges and treatment options. Drug Deliv Transl Res. 2018;8(5):1483–507.
24. Zhang X, Wang J, Hu JM, et al. Synthesis and biological testing of novel glucosylated Epigallocatechin gallate (EGCG) derivatives. Molecules. 2016;21(5):620.
25. Pareja F, Geyer FC, Marchiò C, et al. Triple-negative breast cancer:the importance of molecular and histologic subtyping, and recognition of low-grade variants. NPJ Breast Cancer. 2016;2:16–36.
26. Wein L, Loi S. Mechanisms of resistance of chemotherapy in early-stage triple negative breast cancer (TNBC). Breast. 2017;34(Suppl 1):S27–30.
27. Xue WW, Li WH, Yu Y, et al. Indolocarbazole alkaloid loonamycin A inhibits triple-negative breast cancer cell stemness and Notch signalling. J Pharm Pharmacol. 2023;75(4):523–32.
28. Cheng Y, Lin L, Li X, et al. ADAM10 is involved in the oncogenic process and chemo-resistance of triple-negative breast cancer via regulating Notch1 signaling pathway, CD44 and PrPc. Cancer Cell Int. 2021;21(1):32.
29. Yang CS, Wang H. Cancer preventive activities of tea catechins. Molecules. 2016;21:1679.
30. Luo KW, Lung WY, Xie C, et al. EGCG inhibited bladder Cancer T24 and 5637 cell proliferation and migration via PI3K/AKT pathway. Oncotarget. 2018;9(15):12261–72.
31. Chitnis AB. The role of Notch in lateral Inhibition and cell fate specification. Mol Cell Neurosci. 1995;6:311–21.
32. Krishna BM, Jana S, Singhal J, et al. Notch signaling in breast cancer: from pathway analysis to therapy. Notch signaling in breast cancer: from pathway analysis to therapy. Cancer Lett. 2019;461:123–31.
33. Wang T, Xiang Z, Wang Y, et al. (-)-Epigallocatechin gallate targets Notch to attenuate the inflammatory response in the immediate early stage in human macrophages. Front Immunol. 2017;8:433.
34. Xie H, Sun JQ, Chen YQ, et al. EGCG attenuates uric acid-induced inflammatory and oxidative stress responses by medicating the NOTCH Pathway. Oxid Med Cell Longev. 2015;2015:1–10.
35. Huang YW, Zhu QQ, Yang XY, et al. Wound healing can be improved by (-)-epigallocatechin gallate through targeting Notch in streptozotocin-induced diabetic mice. FASEB J. 2019;33(1):953–64.
36. Wang Y, Shen RW, Han B, et al. Notch signaling mediated by TGF-beta/Smad pathway in Concanavalin A-induced liver fibrosis in rats. World J Gastroenterol. 2017;23(13):2330–6.

## Publisher's note