

Original Research Kaempferol Improves Breast Cancer-Related Depression through the COX-2/PGE2 Pathway

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Abstract

Background: Breast cancer-related depression (BCRD) is strongly associated with BC and increases recurrence and mortality. This study investigated the role of kaempferol in the pathogenesis of BCRD and its underlying mechanism. Methods: 4T1 mouse BC cells were treated with corticosterone (Cort) in vitro to develop a neuronal injury model, and a BCRD mouse model was established by injecting 4T1 cells and Cort. The effects of kaempferol on 4T1 cells and BCRD models were measured by behavioral tests, Cell Counting Kit-8 assay, wound healing assay, colony formation assay, Western blot analysis, quantitative real-time PCR, hematoxylin and eosin staining, enzyme-linked immunosorbent assay, and immunofluorescence. BCRD cells were transfected with the cyclo-oxygenase-2 (COX-2) overexpression plasmid to study the role of the COX-2/prostaglandin E2 (PGE2) axis in the anti-BCRD activity of kaempferol. The connection between kaempferol and COX-2 was analyzed by molecular docking. Results: Kaempferol reduced the viability, migration, and clones of 4T1 cells and inhibited BC growth and depression-like behavior in mice. Kaempferol alleviated inflammation in BCRD, decreased interleukin 1 beta (IL-1 β) and IL-6 levels, and increased transforming growth factor beta 1 (TGF- β 1) and IL-10 levels. In addition, kaempferol elevated the levels of serotonin, dopamine, and norepinephrine and the amount of 5-Bromo-2'-deoxyuridine/neuronal nuclei-positive cells. Kaempferol downregulated COX-2 and PGE2, and kaempferol could dock with the protein structure of COX-2. Overexpression of COX-2 reduced BCRD viability, upregulated $IL-1\beta$ and IL-6 levels, and downregulated $TGF-\beta 1$ and IL-10 expresssion. Overexpression of COX-2 reversed the protective effects of kaempferol. Conclusion: Kaempferol exerted anti-BCRD effects, at least in part by inhibiting the COX-2/PGE2 pathway, which regulates neuroinflammation, neurotransmitter imbalance, and defective neurogenesis. Therefore, kaempferol may be a promising candidate active ingredient for treating BCRD.

Keywords: breast cancer; depression; kaempferol; COX-2; PGE2

1. Introduction

Breast cancer (BC) is the most common cancer among women worldwide, accounting for approximately 25% of new cancer cases in women globally. BC is also the leading of cancer-related death in women [1]. In the process of diagnosis and treatment, in addition to the changes in physical function, BC patients will also bear great psychological pressure. Studies have shown that depression and anxiety are common comorbidities in BC patients, which greatly endanger the quality of life of patients [2]. Metaanalysis showed that depression was associated with relapse and mortality in BC [3]. Therefore, understanding the mechanism behind BC-related depression (BCRD) can help prevent and block cancer development.

It has been reported that neuroinflammation is an important pathological manifestation of depression, and the levels of pro-inflammatory markers are elevated in depressed patients [4]. BC survivors showed more severe depressive symptoms with higher levels of inflammation [5]. Thus, neuroinflammation is expected to be a potential therapeutic direction for BCRD. Cyclo-oxygenase (COX)-2 is expressed in the central nervous system and is closely related to the pathogenesis of various diseases, including degenerative brain diseases, depression, and cancer [6]. COX-2 is usually induced by inflammatory factors and is responsible for regulating the secretion of prostaglandin (PG) E2 during inflammation [7]. Studies have shown that the upregulation of COX-2 and PGE2 can play an anti-neuroinflammation role [8]. Previous reports have described that COX-2 and PGE2 are upregulated in a rat model of depression, and inhibition of the COX-2/PGE2 pathway can reduce the inflammatory response in the hippocampus and effectively improve depressive behavior [9].

Kaempferol is one of the most common flavonoid aglycons in many plants and vegetables. It has been proven that kaempferol has a variety of pharmacological activi-

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ties, including anti-inflammatory, anti-bacterial, neuroprotective, anti-oxidative, anti-diabetic, anti-tumor, and anticancer [10,11]. Among them, kaempferol has been widely recognized as a dietary anti-inflammatory agent [12]. Research has shown that kaempferol has great potential in reducing depression-like behaviors due to its antioxidant and anti-inflammatory effects [13,14]. Additionally, previous studies reported that the administration of kaempferol could reduce the protein abundance of COX-2 in the ischemic stroke model and Parkinson's model [15,16]. However, few reports focus on kaempferol attenuating the progression of BCRD via COX-2/PGE2. Therefore, in this study, we investigated the effects of kaempferol on 4T1 cells, BCRD cells, and mouse models and observed the role of the COX-2/PGE2 pathway.

2. Methods

2.1 Cell Culture and Transfection

The mouse breast cancer cells 4T1 (AW-CCM376) were obtained from Abiowell (Changsha, China) and maintained in Dulbecco's Modified Eagle Medium containing 10% fetal bovine serum and 1% penicillin-streptomycin. The 4T1 cells were treated with 25, 50, and 100 µM kaempferol (N1719; APExBIO Technology LLC, Houston, TX, USA) to investigate the effects of kaempferol [17]. BCRD was mimicked in vitro, as previously described [18]. Briefly, 4T1 cells were stimulated with 10 μ g/mL lipopolysaccharide (Sigma, St. Louis, MO, USA) for 6 h, and the supernatant was collected after centrifugation. The cell supernatant and 200 µM corticosterone (Cort, 50-22-6; Shanghai Aladdin Biochemical Technology Co., Ltd., Shanghai, China) were added to mouse hippocampal neuronal cells HT-22 (AW-CNM116, Abiowell) and cultured for 6 h to obtain BCRD cells. Then the BCRD cells were treated with kaempferol. Cells were divided into Control, BCRD, and Kaempferol groups. 4T1 cells and HT-22 cells were tested for mycoplasma. Through short tandem repeat (STR) detection, it was verified that the cell line we used was 4T1 and HT-22 cells, and there was no humansource pollution. The 4T1 (https://www.cellosaurus.org/ CVCL 0125) and HT-22 cells (https://www.cellosaurus.or g/CVCL_0321) used in this study were checked and found no cross-contamination.

The overexpression (oe) plasmids, oe-NC and oe-COX-2(HG-MO011198) (both from Honor Gene Co. Ltd., Changsha, China), were transfected into BCRD cells using Lipofectamine 2000 reagent (11668019; Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The sequence of COX-2 was obtained from the National Center for Biotechnology Information, and primers were designed to isolate the target gene. The target fragment was ligated into the pcDNA3.1(+) vector. After being stimulated with 25 μ M kaempferol for 48 h, oe-NC and oe-COX-2 were transfected into BCRD cells. Cells

were divided into BCRD, oe-NC, oe-COX-2, Kaempferol, Kaempferol+oe-NC, and Kaempferol+oe-COX-2 groups.

2.2 Cell Counting Kit-8 (CCK8) Assay

To assess cell viability, we used the Cell Counting Kit-8 (CCK8) assay. In brief, after pancreatic enzyme digestion, cells in the logarithmic growth phase were seeded in a 96-well plate at a density of 5×10^3 cells/well in a volume of 100 µL per well. After drug treatment, 10 µL CCK-8 reagent (AWC0114a, Abiowell) was added to each well, thoroughly mixed, and incubated for 4 h. A microplate reader (MB-530, Shenzhen Heales Technology Development Co., Ltd., Shenzhen, China) was used to measure the absorbance at 450 nm. Each group had at least three replicates.

2.3 Wound-Healing Assay

The wound healing assay was performed to assess cell migration. After digestion with trypsin, 4T1 cells were seeded in a 6-well plate at a density of 5×10^5 cells/well in a volume of 1 mL per well. Subsequently, a sterile pipette tip was used to streak vertically. The scratched cells were washed away with phosphate-buffered saline (PBS). Then, the complete medium was replaced with a serum-free medium to limit the effects of cell proliferation [19], and cells were cultured at 37 °C for 48 h. Images were taken at 0 h, 24 h, and 48 h, and the width value of the area not covered by cells (width value, μ m) was recorded.

2.4 Colony Formation Assay

The colony formation assay was used to analyze cell proliferation. After kaempferol treatment, 4T1 cells (200 cells/mL) were seeded in 6-well plates and dispersed evenly. Cells were allowed to grow for the next 14 d to form colonies. After terminating the culture, the cells were fixed in 4% paraformaldehyde for 15 min, stained with crystal violet dye (AWC0333; Abiowell), and counted.

2.5 BCRD Animal Model

Six-week-old female BALB/c mice were purchased from Hunan SJA Laboratory Animal Co., Ltd. (Changsha, China). As previously described, 4T1 cells (1 \times 10^6 cells/100 µL) were injected into the right mammalian fat pads of mice to establish a BC model [20]. Tumor formation was observed after 7 days. Subsequently, a mouse model of BCRD was established by administering 30 mg/kg Cort daily through subcutaneous injection in the back of BC mice for 21 days. The experimental groups included Control, BCRD, and Kaempferol groups (n = 5). The Kaempferol group additionally received 20 mg/kg/d kaempferol (HY-14590; MedChemExpress, Monmouth Junction, NJ, USA) by intraperitoneal injection for 21 d [14,17]. The Control group was treated with the same dose of normal saline. The survival rate of mice remained at 100% throughout the modeling process.

2.6 Open Field Test (OFT)

To assess anxiety, the open field test (OFT) was conducted. Briefly, mice were quickly placed in the center of a square experimental box ($50 \times 50 \times 40$ cm), and the mice were allowed to move freely. During 15 min of training, the total distance traveled by the mice was recorded [21].

2.7 Forced Swimming Test (FST)

To assess depressive-like states, the forced swimming test (FST) was performed. Briefly, mice were placed in clear glass vials that were 21 cm high and 16.5 cm in diameter. Water about 13 cm deep was added to the bottle, and the mice were forced to swim for 6 min. The behavior of the mice was observed for 4 min, and the immobility time was recorded. Mice were considered stationary when their head was above the water's surface with no apparent struggle [22].

2.8 Tail Suspension Test (TST)

For the tail suspension test (TST), mice (1 cm from the tail tip) were suspended in the air with adhesive tape, about 60 cm above the ground. Mice were separated from each other. During the 6 min test period, the activity of the mice was observed, and the immobility time within the next 4 min was recorded [23].

2.9 Hematoxylin-Eosin (HE) Staining

Hematoxylin and eosin (HE) staining was used to assess the degree of pathological damage. Tumors from BCRD mice were collected and then subjected to fixing, embedding, and sectioning (4 μ m). Sections were placed in xylene for 20 min and repeated three times. Then, the sections were hydrated through different gradients of ethanol (75–100%) and washed with distilled water. Hematoxylin (AWI0009; Abiowell) and eosin (AWI0020, Abiowell) were used for staining. Sections were dehydrated in graded alcohol (95%–100%) and placed in xylene. The morphological changes were observed and photographed using a microscope (BA210T; Motic Microscopes, Xiamen, China).

2.10 Quantitative Real-Time PCR (qRT-PCR)

Total RNAs from cells were extracted with TRIzol (15596026; Thermo Fisher Scientific, Waltham, MA, USA) and reversely transcribed to prepare cDNAs. The relative expression of targets was performed by the UltraSYBR Mixture kit (CW2601; ConWin Biosciences, Taizhou, China) and normalized to β -actin. The relative mRNA enrichment of targets was determined with the $2^{-\Delta\Delta Ct}$ method. The primers are listed in Table 1.

2.11 Western Blot

Total protein from mice hippocampal tissues or cell lysates was extracted with RIPA (AWB0136, Abiowell). After protein quantification with a BCA kit (AWB0104,

	Table 1. Primer sequences.
Gene	Sequences (5'-3')
IL-1β	F: TGAAATGCCACCTTTTGACAGT
	R: TTCTCCACAGCCACAATGAGT
IL-6	F: GACTTCCATCCAGTTGCCTT
	R: ATGTGTAATTAAGCCTCCGACT
TGF-β1	F: CTCCCGTGGCTTCTAGTGC
	R: GCCTTAGTTTGGACAGGATCTG
IL-10	F: GTTCCCCTACTGTCATCCCC
	R: AGGCAGACAAACAATACACCA
COX-2	F: AATACTGGAAGCCGAGCACCT
	R: ACACCCCTTCACATTATTGCAGA
PGE2	F: TCTATGGGGCCTCCTTGCTCT
	R: AGCACAGCCACGATAAGCAG
β -actin	F: ACATCCGTAAAGACCTCTATGCC
	R: TACTCCTGCTTGCTGATCCAC

Abiowell), proteins were resolved by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and then electrotransferred to nitrocellulose membranes. After blocking in 5% skimmed milk for 90 min, the membranes were incubated overnight at 4 °C with the following primary antibodies: COX-2 (1:2000, 12375-1-AP; Proteintech Group Inc., Rosemont, IL, USA), PGE2 (1:300, ab217966; Abcam, Cambridge, MA, UK), interleukin 1 beta (IL-1 β) (1:2000, 16806-1-AP; Proteintech), IL-6 (1:1000, ab233706; Abcam), transforming growth factor beta 1 (TGF- β 1) (1:1000, ab31013; Abcam), IL-10 (1:1000, 20850-1-AP; Proteintech), and β -actin (1:5000, 66009-1-Ig; Proteintech). Then the membranes were washed with PBS with Tween 20 (PBST), followed by incubation with anti-mouse (1:5000, SA00001-1; Proteintech) and anti-rabbit (1:6000, SA00001-2; Proteintech) secondary antibodies for 90 min and washing with PBST. ECL Plus Substrate (AWB0005; Aiowell) and a gel imaging system (ChemiScope 6100; Clinx, Shanghai, China) were used to visualize the proteins. Protein expression levels were quantified by scanning densitometry using Quantity one 4.6 software (Bio-Rad Laboratories, Inc., Hercules, CA, USA).

2.12 Enzyme-Linked Immunosorbent Assay (ELISA)

According to the manufacturer's instructions, the levels of serotonin (5-HT, CSB-E08365m), dopamine (DA, CSB-E08661m), norepinephrine (NE, CSB-E07870m), IL-1 β (CSB-E08054m), IL-6 (CSB-E04639m), TGF- β 1 (CSB-E04726m), IL-10 (CSB-E04594m), COX-2 (CSB-E12910m), and PGE2 (CSB-E07966m) were measured. All kits were from Cusabio Biotech Co., Ltd. (Wuhan, China).

2.13 Molecular Docking

The two-dimensional (2D) structure of COX-2 was downloaded from the Protein Data Bank (https://www.rc

sb.org/), and the 3D structure of kaempferol was obtained from the PubChem database (https://pubchem.ncbi.nlm.nih .gov/compound/5280863). Autodock Vina software was performed to analyze the interaction of kaempferol and COX-2, and Discovery Studio was used for analyzing and viewing the 2D and 3D images.

2.14 Immunofluorescence (IF)

Mouse brain tissue underwent embedding and sectioning. After deparaffinization and hydration, slices were placed in an EDTA buffer and heated for thermal antigen retrieval. After cooling, the slices were washed with PBS. Then sections were sequentially incubated in sodium borohydride, 75% ethanol, and Sudan black solution. Slices were blocked in 5% bovine serum albumin for 1 h, then incubated with 5-Bromo-2'-deoxyuridine (BrdU) (1:50, ab8152; Abcam) and neuronal nuclei (NeuN) (1:50, 26975-1-AP; Proteintech) primary antibodies overnight at 4 °C. Subsequently, the slices were incubated with anti-rabbit (1:500, SA00013-2; Proteintech) or anti-mouse (1:500, SA00013-3; Proteintech) secondary antibodies at 37 °C for 1 h. Slices were counterstained with DAPI for 10 min and then imaged and photographed under a fluorescent microscope.

2.15 Statistical Analysis

Statistical analysis was conducted using GraphPad Prism 9.0 software (GraphPad Software Inc., San Diego, CA, USA). Data are presented as the mean \pm standard deviation. Data from two samples were compared using the Student's *t*-test. Analysis of variance (ANOVA) was used to compare data from multiple groups. All experiments included three biological replicates. p < 0.05 was considered statistically significant.

3. Results

3.1 Kaempferol Inhibits 4T1 Cell Viability, Migration, and Colony Formation

To assess the effects of kaempferol on BC, 4T1 cells were stimulated with different concentrations of kaempferol (25, 50, and 100 μ M). The results showed that after treatment for 24 and 48 h, kaempferol significantly reduced the viability of 4T1 cells, and the inhibitory ability was gradually increased in a dose-dependent manner (Fig. 1A). The wound healing assay showed that after 24 h and 48 h, kaempferol alleviated the migration of 4T1 cells in a dose-dependent manner (Fig. 1B,C). In addition, the number of cloned 4T1 cells was reduced after kaempferol intervention, and the effect of 100 μ M kaempferol was the most prominent (Fig. 1D). These results showed that kaempferol inhibited 4T1 cell proliferation, and colony formation.

3.2 Kaempferol Improves Inflammation in Hippocampal Neurons

Next, the effects of kaempferol on inflammation in neuronal cells were examined. The non-toxic dose of

kaempferol was determined by the CCK-8 assay. Compared with the Control group, BCRD cells showed decreased cell viability, whereas the cell viability was partially restored after stimulation with different concentrations of kaempferol (10 µM, 25 µM, and 50 µM). Notably, in BCRD cells, when the dose of kaempferol reached 50 μM, the cell viability showed a downward trend (Fig. 2A). Therefore, we applied 25 µM kaempferol for experiments. Subsequently, the levels of inflammatory factors were detected. BCRD cells showed increased levels of proinflammatory cytokines (*IL-1\beta* and *IL-6*), and kaempferol reversed this trend (Fig. 2B,C). Meanwhile, kaempferol increased the levels of anti-inflammatory factors (TGF- $\beta 1$ and *IL-10*) levels in BCRD cells (Fig. 2D,E). In addition, kaempferol inhibited the protein accumulation of COX-2 and PGE2 in BCRD cells (Fig. 2F). Taken together, these results showed that kaempferol attenuated inflammatory manifestations effects and downregulated COX-2 and PGE2 in BCRD cells.

3.3 Kaempferol Reduces Neuroinflammation, at Least in Part through the COX-2/PGE2 Pathway

The role of COX-2/PGE2 in the anti-inflammatory effect of kaempferol was further explored. To confirm the kaempferol-COX-2 interaction, molecular docking was selected for analysis. Kaempferol and COX-2 had a binding energy of -9.1 kcal/mol, which indicated a strong and stable interaction between kaempferol and COX-2. In the visualized images, kaempferol bound to specific residues within the active pocket of COX-2, including PRO127, TYR2373, ARG376, GLN374, GLN2374, PHE142, PRO2538, GLY2227, VAL2228, GLY2533, ASN2537, TRP139, GLY2536, ASN-2375, GLY2225, ARG2376, and LEU2145. More specifically, kaempferol formed a Pi-Sigma conjugation at the GLN2374 residue. Kaempferol formed an Amide-Pi stacked at residue GLY2536. Kaempferol formed an unfavorable Donor-Donor at the ASN2537 residue. Kaempferol formed Pi-Alkyl and carbon-hydrogen bonds at the PRO2538 Kaempferol formed Pi-Donor hydrogen and residue. conventional hydrogen bonds at the ASN2375 residue. Kaempferol formed a conventional hydrogen bond at residues GLN374, VAL2228, and ARG2376. Furthermore, kaempferol formed van der Waals at residues ARG376, TYR2373, PRO127, PHE142, GLY2227, GLY2533, TRP139, GLY2225, LEU2145. These results suggested that kaempferol can bind to COX-2 (Fig. 3A).

Next, BCRD cells were transfected with the overexpression plasmid oe-COX-2. Our results showed that compared with the oe-NC group, oe-COX-2 increased the expression of both COX-2 and PGE2 in BCRD cells. By contrast, treatment with kaempferol reduced the expression of COX-2 and PGE2 in BCRD cells compared with the BCRD group. Compared with Kaempferol+oe-NC, oe-COX-2 elevated the expression of COX2 and PGE2



Fig. 1. Kaempferol reduced 4T1 cell viability, migration, and colony formation. (A) 4T1 cell viability was detected by the cell counting kit-8 (CCK8) assay at 24 h and 48 h. (B,C) The wound healing assay was performed to measure 4T1 cell migration at 24 h and 48 h. Areas not covered by 4T1 cells were measured and counted. (D) Cell numbers were counted to assess 4T1 cell clonality. n = 3. * p < 0.05 vs. Control.

in BCRD cells after kaempferol intervention. Compared with the oe-COX-2 group, kaempferol inhibited the expression of COX2 and PGE2 (Fig. 3B,C). In addition, oe-COX-2 decreased BCRD cell viability compared to the oe-NC group, whereas Kaempferol enhanced BCRD cell viability. The Kaempferol+oe-COX-2 group exhibited re-

duced BCRD cell viability compared to Kaempferol+oe-NC. Kaempferol decreased cell viability compared with the oe-COX-2 group (Fig. 3D). Compared with the oe-NC group, oe-COX-2 increased *IL-1* β and *IL-6* levels and decreased *TGF-* β *1* and *IL-10* levels. Compared with the BCRD group, kaempferol downregulated *IL-1* β and *IL-6*

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Fig. 2. Kaempferol regulated the expression of inflammatory cytokines and cyclo-oxygenase (COX)-2/PGE2. (A) The effect of kaempferol (10 μ M, 25 μ M, and 50 μ M) on cell viability was assessed at 24 h and 48 h. The mRNA levels of *IL-1* β (B), *IL-6* (C), *TGF-* β *1* (D), and *IL-10* (E) were measured. (F) The abundance of COX-2 and PGE2 was examined. n = 3. * p < 0.05 vs. Control, # p < 0.05 vs. breast cancer-related depression (BCRD).

and upregulated $TGF-\beta I$ and IL-10. oe-COX-2 increased the kaempferol-decreased $IL-1\beta$ and IL-6 levels and attenuated the kaempferol-increased $TGF-\beta I$ and IL-10 levels compared to Kaempferol+oe-NC. Compared with the oe-COX-2 group, the Kaempferol+oe-COX-2 group showed decreased $IL-1\beta$ and IL-6 levels and increased $TGF-\beta I$ and IL-10 levels (Fig. 3E). These results suggested that COX-2 overexpression inhibited BCRD cell viability and regulated the expression of inflammatory factors. Thus, kaempferol may have a therapeutic role in neuroinflammation, at least in part, by targeting COX-2/PGE2.

3.4 Kaempferol Alleviates Pathological Damage in BCRD Mice

We developed a mouse model of BCRD to confirm the therapeutic effect of kaempferol *in vivo*. Compared with the BCRD group, kaempferol promoted the growth of mice (Fig. 4A). Compared with the BCRD group, kaempferol significantly inhibited the volume and weight of BC tumors (Fig. 4B,C), suggesting that kaempferol had certain anti-BC effects on BCRD mice. Next, the tumor structure of BC was observed. As shown in Fig. 4D, the tumors of kaempferoltreated BCRD mice showed reduced inflammatory cell infiltration, clear cell structure, and tight arrangement, showing that kaempferol protected the pathological tumor structure of BCRD mice.

3.5 Kaempferol Relieves Depressive Behavior in BCRD Mice

The effect of kaempferol on the depressive behavior of mice in the BCRD group was assessed by behavioral studies. The results of the FST showed that the immobility time of BCRD mice increased, and kaempferol effectively reduced the immobility time of BCRD mice (Fig. 5A). The OFT showed that the total distance of mice in the BCRD group was significantly reduced, but kaempferol reversed this trend (Fig. 5B). Kaempferol inhibited the stress-induced increase in immobility time during the TST (Fig. 5C). Major neurotransmitters (NTs) in depression include (5-hydroxy tryptamine, 5-HT), dopamine (DA) and norepinephrine (NE). Compared with the Control group, 5-HT, DA, and NE levels were downregulated in the BCRD group, while kaempferol partially restored their levels (Fig. 5D). Taken together, these findings demonstrated that kaempferol contributed to reducing depression-like behaviors in BRCD mice.



Fig. 3. Kaempferol inhibited the COX-2/PGE2 pathway to relieve neuroinflammation. (A) Molecular docking revealed the binding of kaempferol to COX-2. (B) The mRNA and protein levels of COX-2 were measured. (C) The mRNA and protein levels of PGE2 were measured. (D) The effect of COX-2 on cell viability was detected. (E) The effects of COX-2 on the levels of *IL-1* β , *IL-6*, *TGF-* β 1, and *IL-10* were examined. n = 3. & p < 0.05 vs. oe-NC, * p < 0.05 vs. BCRD, #p < 0.05 vs. Kaempferol+oe-NC.



Fig. 4. Kaempferol inhibited the growth of BC and neuroinflammation in BCRD mice. (A) Within 28 days of modeling, the mouse weight in each group (n = 5) was recorded. (B) Within 28 days of modeling, the tumor growth in each group was recorded. (C) On the 28th day, the tumors of mice in each group were photographed and weighed. (D) Representative images of the pathological phenotype in each group were presented. n = 3. * p < 0.05 vs. BCRD.

3.6 Kaempferol Protectes BCRD Mice from Neuronal Damage at least in part via the COX-2/PGE2 Signaling

IL-1 β and IL-6 levels in the peripheral blood of mice in the BCRD group were increased, and TGF- β 1 and IL-10 levels were decreased, whereas kaempferol reversed the levels of these cytokines (Fig. 6A). The protein abundance of these cytokines in the hippocampus of BCRD mice was also restored by kaempferol (Fig. 6B). Moreover, peripheral blood and hippocampal tissues of BCRD mice showed increased COX-2 and PGE2 expression, which were effectively interfered with by kaempferol (Fig. 6C,D). BrdU/NeuN staining revealed that kaempferol reversed the reduction of BrdU/NeuN neurons in BCRD mice (Fig. 6E). Our results suggested that kaempferol improved the development of BCRD at least in part by inhibiting COX-2/PGE2.

4. Discussion

Compared with healthy women, BC patients have a significantly increased risk of depression, and identifying depression can help improve the mental status and quality of life in BC patients [24,25]. Studies have shown that depression has a pathogenic effect on BC, so adjuvant therapy for depression has become a potential direction for the prevention and treatment of BC [20,26]. Currently, medication, electroconvulsive therapy, and cognitive-behavioral therapy are commonly used to treat mental illnesses, including depression [27]. Due to drug interactions or significant toxicity, the tolerance is reduced, and the therapeutic effect is not ideal. The development of natural products of plant origin may be a safer and more effective alternative therapy [28]. Currently, the role of some natural products in the treatment of depression has been discovered, such as Isoliquiritin [29], Baicalin [30], and Kaempferol [31]. In



Fig. 5. Kaempferol improved depression in BCRD mice. (A) Forced swimming test (FST). (B) Open field test (OFT). (C) Tail suspension test (TST). (D) The contents of 5-hydroxy tryptamine (5-HT), dopamine (DA), and norepinephrine (NE) were examined by ELISA. n = 3. * p < 0.05 vs. Control, # p < 0.05 vs. BCRD.

addition, kaempferol is reportedly a therapeutic agent for BC [32]. Therefore, we speculated that kaempferol could be used to treat BCRD.

In our study, kaempferol downregulated the viability, migration, and clone of 4T1 cells. After kaempferol treatment, tumor growth in BCRD mice was significantly alleviated. In behavioral tests, BCRD mice lost interest in exploring novel environments and had increased inactivity time during the TST and FST, which was reported in our previous study [20]. Conversely, kaempferol effectively increased the total journey and decreased the immobility time in BCRD mice. These results suggest that kaempferol has a certain therapeutic effect on BCRD. Neuroinflammation is widely recognized to be involved in the pathology of depression [33]. Improvement of neuroinflammation can re-

verse depressive behavior in many cases involving inflammatory factors fluctuations. Our study showed that IL-1 β and IL-6 levels were increased, and TGF- β 1 and IL-10 levels were decreased in the peripheral blood and hippocampus of BCRD mice, whereas kaempferol reversed the trend. Kaempferol reduced the inflammatory response of the body to the tumor in BCRD mice. In addition, depression is manifested by neurotransmitter imbalances and neurogenesis deficits [34]. In BCRD mice, kaempferol partially restored neuronal proliferation and increased 5-HT, DA, and NE levels, mediating neurogenesis and neurotransmitters. Taken together, our data showed that kaempferol attenuated the development of BCRD by modulating neuroinflammation, neurogenesis, and neurotransmitters.



Fig. 6. Kaempferol improved neuronal damage in BCRD mice, at least in part through COX-2/PGE2 signaling. The levels of IL-1 β , IL-6, TGF- β 1, and IL-10 in the peripheral blood (A) and hippocampal tissues (B) of mice in each group were examined. The levels of COX-2 and PGE2 in peripheral blood (C) and hippocampal tissues (D) of mice in each group were measured. (E) Immunohistochemistry staining was applied for the detection of hippocampal neurogenesis. 5-hydroxy tryptamine (BrdU)/neuronal nuclei (NeuN) positive cells were calculated. n = 3. * p < 0.05 vs. Control, #p < 0.05 vs. BCRD.

COX-2 is an inflammatory mediator, and COX-2 inhibitors are considered to be promising anti-inflammatory and antidepressant drugs [35,36]. Previous studies have shown that curcumin may exert antidepressant effects, at least in part by limiting COX-2 signaling to modulate factors such as postsynaptic transmission and cell viability [37]. Another study demonstrated that puerarin reduces COX-2 expression, thus helping to restore intestinal mucus barrier dysfunction and neuroinflammatory hyperactivation and ultimately mitigating depression-like behavior [38]. In the present study, we demonstrated that the abundance of COX-2 was increased in the BCRD model. Kaempferol restricted the expression of COX-2, which is consistent with previous reports [39]. Overexpression of COX-2 decreased the viability of BCRD cells, upregulated $IL-1\beta$ and *IL-6*, downregulated *TGF-\beta1* and *IL-10*, and blocked the anti-inflammatory activity of kaempferol. The excessive secretion of inflammatory factors will lead to a 5-HT level imbalance, causing an imbalance of neurotransmitters [40]. Overexpression of COX-2 leads to an increase in proinflammatory cytokines, affects 5-HT, HA, and DA signaling pathways, and further regulates the pathogenesis of depression [8]. In addition, studies have shown that inflammatory factors and COX-2 are closely related to hippocampal neurogenesis, thereby affecting depression-like behaviors [41]. Notably, our analysis showed that kaempferol could bind to COX-2, suggesting that kaempferol directly targeted the COX-2 signaling to play a role in the treatment of BCRD. These findings suggest that kaempferol may serve as a natural COX-2 inhibitor, exerting anti-BCRD effects.

A growing number of reports have proposed that blocking COX-2 activity improves stress-induced depression-like behavior, a process involving decreasing PGE2 [36]. COX-2 is a rate-limiting enzyme responsible for the final conversion of arachidonic acid (AA) to PGE2 [42]. More specifically, AA is converted to the unstable intermediate prostaglandin H2 (PGH2) via COX, which is further converted to PGE2 by terminal PGE2 synthases (PGES). After synthesis, PGE2 binds to receptors known as prostaglandin E receptors (EP1-4), which are coupled to G proteins and participate in the regulation of inflammation [43,44]. Therefore, it appears that COX-2 catalyzes the production of PGE2, and PGE2 then binds to receptors to activate downstream signaling pathways. Due to financial and time constraints, the more intricate regulatory connection between COX-2 and PGE2 remains to be further explored, which will be further investigated in our future studies. Our report showed that the abundance of PGE2 was increased in the BCRD model. Kaempferol and overexpression of COX-2 effectively decreased its expression, suggesting that kaempferol inhibited BCRD progression, at least in part through the COX-2/PGE2 signaling pathway. COX-2 exerts most of its functions through its metabolite PGE2 and is also involved in



various signaling pathways such as nuclear factor kappa B, extracellular signal-regulated kinase, phosphoinositide 3-kinase/AKT, and cyclic AMP/protein kinase A to modulate neurological function [45,46]. COX-2 plays a critical role in depression by regulating gut microbiota, mitochondrial function, hippocampal neuronal damage, and the hypothalamic-pituitary-adrenal axis [8]. Therefore, further investigation is needed to elucidate the mechanisms by which kaempferol ameliorates BC development and depression-like behaviors via COX-2. Additionally, the development of BCRD has been linked to intestinal flora dysbiosis, neuronal pyroptosis, and immune responses [18,47]. However, whether kaempferol targets other biological processes to regulate BCRD progression still needs more experimental evidence.

5. Conclusion

To summarize, our study supported the role of kaempferol in anti-BCRD, at least in part through the COX-2/PGE2 signaling pathway, to regulate neuroinflammation, neurogenesis, and neurotransmitters. Our findings broaden kaempferol-based therapeutic strategies and provide potential directions for treating BCRD.

Availability of Data and Materials

The datasets used during the current study are available from the corresponding author on reasonable request.

Author Contributions

YS designed the research study. QZ, YHa, YHe, YF, and HY performed the research. YHa and YC analyzed the data. QZ wrote the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work to take public responsibility for appropriate portions of the content and agreed to be accountable for all aspects of the work in ensuring that questions related to its accuracy or integrity.

Ethics Approval and Consent to Participate

Animal experiments were approved by the Ethic Committee of Hunan Cancer Hospital & The Affiliated Cancer Hospital of Xiangya School of Medicine, Central South University (No. SBQLL-2021-034).

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Conflict of Interest

The authors declare no conflict of interest.

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