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The effect of curcumin on breast cancer cell viability, apoptosis, and the NF-kB Signaling Pathway

Yongheng Xie

Chengdu Foreign Language School, Chengdu, 611731, China
billyheng2007@163.com

Abstract:

Breast cancer is the most prevalent form of cancer among women after skin cancer in the United States. In 2022, 2.3 million women were diagnosed with breast cancer, and 670000 people died. Curcumin, one of the natural compounds that originated from India, has been found to have potential anti-cancer properties by interacting with multiple signaling pathways. The purpose of this work is to examine the impacts of curcumin therapy on apoptosis, the proliferation of breast cancer cells, and therapeutic approaches that target the NF-kB system. The MCF-7 is a popular cell line for breast cancer research. We will employ the MTT assay and flow cytometry to evaluate the impacts of curcumin on cancer cell viability and apoptosis. Western blot will be used to determine whether curcumin decreases phosphor-IkB, which indicates the inhibition of gene expression. The findings will provide a better understanding of the effects of curcumin. Future research should focus on different cell signaling pathways that contribute to cell survival or cell death and various cell lines treated by curcumin. The purpose is to test that Curcumin can modulate the NF-κB signaling pathway, which in turn reduces the proliferation and promotes apoptosis in MCF-7 breast cancer cells.

Keywords: MCF-7 cells; Curcumin; NF-kB pathway

1. Introduction

Breast cancer, a disease where abnormal breast cells grow out of control, has been considered to be one of the most common cancers in the United States[1]. Cancer cells proliferating throughout the body and eventually becoming lethal is the main mechanism behind the growth of breast tumors. The milk ducts or lobules that produce breastmilk are the source of breast cancer cells. The earliest form is not fatal and can be identified in its early stages. Carcinogenic cells may infect nearby breast tissue. This results in tumors that swell or become lumpy. Breast cancer is classified into stages from 0 to IV. Stage 0 involves cancer cells confined to ducts without invasion. Stage I indicates that cancer has spread to other tissue in

a small area. Stage II involves larger tumors or limited spread to nearby lymph nodes. Stage III denotes a tumor greater than 50 mm and involving more lymph nodes in a broader area. Stage IV indicates that the tumor can grow any size and the disease has spread to other tissues and organs. Cancer may have spread to the skin or chest wall. Cancer has spread beyond the breast to other parts of the body. From 2014 to 2018, the incidence of breast cancer in the United States had a slow increase for female breast cancer of 0.5% annually[2]. Currently, surgery (lumpectomy or mastectomy) has been developed as low-risk procedure for the treatment of breast tumors, but both procedures of lumpectomy and mastectomy may lead to a seroma, hematoma, infection, skin necrosis, or lymphedema. In addition, Chemotherapy is an alternative to surgery as a treatment for breast cancer. However, chemotherapy not only kills uncontrollable growing cancer cells but also damages the healthy cells that grow and divide quickly. It may cause fatigue, nausea, vomiting, and loss of appetite.

Curcumin (Figure 1), a vivid yellow substance generated by Curcuma longa plants, has been thoroughly studied by scientists from both a biological and chemical perspective. Turmeric, the source of curcumin, is a rhizomatous herbaceous perennial plant (Curcuma longa) of the ginger family[3]. The antibacterial, anticancer, and antioxidant properties of curcumin have led to its current use[4]. It can modulate diverse transcription factors, inflammatory cytokines, enzymes, receptors, growth factors, and kinases. Furthermore, curcumin effectively regulates tumor cell growth via regulating various cell signaling pathways[5,6].

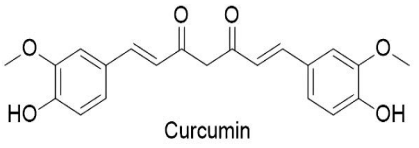

Figure 1. The structure of curcumin[6]

A family of inducible transcription factors known as nuclear factor-kB (NF-kB) regulates the transcription of DNA, the generation of cytokines, and cell viability. Canonical and noncanonical are two major signaling pathways triggered by NF-kB. NF-kb family includes p105/p50, p100/p52, p65/RelA, RelB, and c-Rel. The heterodimer of RelA and p50 is essential in the transcription of NF-kB when the canonical pathway is active, whereas in the noncanonical pathway, p52 and RelB form a heterodimer[7]. The NF-kB proteins are inhibited by the inhibitor proteins found in members of the IkB family and similar proteins that include ankyrin repeats[8]. Tumor stroma cells and cancerous cells proliferate when NF-kB is activated, and this results in the production of cytokines like IL-1β and IL-6. Figure 2 shows the NF-kB pathway.

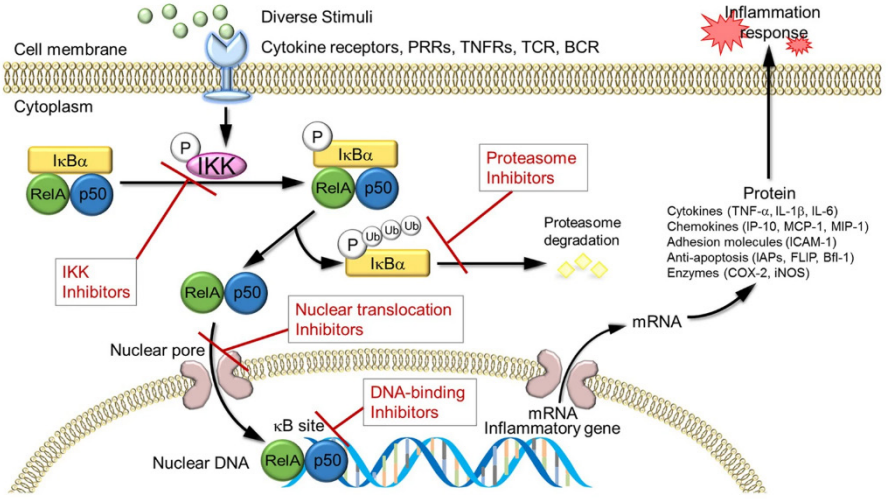

Figure 2. The NF-kB pathway[9]

MCF-7 is a commonly utilized cell line for breast cancer. The MCF-7 cells are distinguished by their ER-positive status. They were obtained from the pleural effusion of a 69-year-old woman with malignant breast cancer [10].

In this study, we will use the MCF-7 cell line to investigate the effect of curcumin on the proliferation and death of MCF-7 breast cancer cells mediated by the NF-kB signaling system.

2. Hypothesis

Curcumin is expected to modulate the NF-κB signaling pathway, which in turn reduces the proliferation and promotes apoptosis in MCF-7 breast cancer cells.

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3. Method and Materials

3.1 Cell Line

The curcumin purchased from Merck KGaA will be dissolved in dimethyl sulfoxide (DMSO) to a concentration of 10mM and stored at -20°C. For treatment groups, MCF-7 cells will be administrated with gradient concentrations of curcumin (1, 5, 10, 20, 40, 80, and 160 μM) for time intervals (24, 48, and 72 hours). Doxorubicin will be used as a positive control, prepared similarly with appropriate dilutions. PBS/DMSO will be used as a negative control which will also be prepared similarly with appropriate dilutions.

3.2 Method 1 Effect of Curcumin on cell viability by MTT assay

The MCF-7 human cell line purchased from the European Collection of Authenticated Cell Cultures will be used in this experiment to examine whether curcumin reduces viability using the MTT assay. The MCF-7 cells will be grown in a complete medium with 10% fetal bovine serum at 37°C in an incubator with 5% CO2 humidification. The MCF-7 cells will be seeded into 96-well plates at a density of 1 × 105 cells/mL in triplicates and allowed to adhere overnight. Following a 24-hour period, the cells will be subjected to different curcumin concentrations (1, 5, 10, 20, 40, 80, and 160 μM) for a duration of 72 hours, and 0.1% DMSO will be added to the control wells[11]. The plates are going to be incubated for 4 hours at 37°C with 5% CO2 with 20 μL of a 5 mg/mL MTT solution, which will be prepared in PBS and filtered through a 0.2 μm filter)[12]. After removing the MTT solution, 200 μL of DMSO will be added in order to dissolve the violet formazan crystals[13]. Using a VersaMax microplate reader (Molecular Devices, LLC) and a reference wavelength of 630 nm, the optical density (OD) of the plates will be measured at 570 nm after five minutes of shaking them. The percentage of DMSO-treated cells will be used to compute the vitality of the cells, and the inhibition curve will be used to calculate the curcumin IC_{50} value, which will be displayed as the three independent studies' mean ± standard deviation.

3.3 Method 2 Curcumin increases AnnexinV/PI by FACS

The MCF-7 human cell line purchased from Thermo Fisher Scientific will be used in this experiment to examine the apoptosis by flow cytometry. The MCF-7 cells will be cultured at 37°C in an incubator with 5% CO2 humidification in a complete medium with 10% fetal bovine serum.

For a 6-well plate, the MCF-7 cells will be plated at a density of 2×10^5 cells/well and incubated at 37°C. After adhesion, the cells will be exposed to different curcumin concentrations (1, 5, 10, 20, 40, 80, and 160 μM) for a duration of 48 hours. 0.1% DMSO will be added to the control wells. Trypsin will be used to separate the cells after treatment, and they will then be rinsed with ice-cold PBS. After that, the cells shall be reconstituted in chilled 1× binding buffer[11]. Annexin V-FITC and Propidium Iodide will be added for staining, and the mixture will be treated for 15 minutes at ambient temperature in the dark[14]. The cells will be analyzed using a FACSCalibur flow cytometer[15].

3.4 Method 3 Curcumin decreases phospho-IkB by western

The MCF-7 cell line will be used in this experiment to examine the decrease in phospho-IkB by western blot. The MCF-7 cell line will be acquired from Sigma-Aldrich and cultivated at 37°C in a moist atmosphere with 5% CO2 in a complete medium containing 10% fetal bovine serum.
In 6-well plates, MCF-7 cells will be planted at a density of 5×10^5 cells per well, and they will be left to adhere for the entire night. Following a 12-hour curcumin treatment with varying concentrations, cells will be collected using RIPA buffer, chilled for half an hour, and centrifuged at 12,000 × g for 15 minutes at 4°C[11]. The protein concentrations will be quantified using the BCA protein quantitation reagent kit. Equal amounts of protein from each sample will be mixed with 2× SDS-PAGE loading buffer. The proteins from each sample will be boiled for 10 minutes and resolved on a 10% SDS-PAGE gel. Proteins will be transferred to nitrocellulose membranes using a transfer buffer for 2 hours[16]. Following an hour at room temperature in TBST with 5% non-fat milk to inhibit membranes, primary antibodies against NF-κB will be incubated on them for an additional night at 4°C. Following three 10-minute TBST washes, membranes will be incubated for one hour with horseradish peroxidase-conjugated secondary antibodies and then washed one more time. Protein bands will be visualized using the ECL Plus Western Blotting Detection system and quantified using Image Pro Plus 5 software[17].

4. Statistical Analysis

In the statistical analysis, the three groups will be compared using a one-way analysis of variance (ANOVA). For statistical significance, a p-value of less than 0.05 was considered. For all of the analyses, SPSS software version 13.0 (SPSS, Inc., Chicago, IL, USA) will be utilized. Every experiment ought to be carried out three times, with

varying curcumin concentrations and durations.

5. Results

Table 1. Possible combinations of results

Combination Result # (CR#)	Curcumin decreases viability by MTT assay?	Curcumin increases AnnexinV/PI by FACS?	Curcumin decreases phospho-IkB by western	Support of hypothesis
1	+	+	+	Full
2	+	+	-	Partial
3	+	-	+	Partial
4	-	+	+	Partial
5	+	-	-	Partial
6	-	+	-	Partial
7	-	-	+	Partial
8	-	-	-	Fully Contradicts

Table legend: "+" indicates that the phenomenon in each column heading is observed in the experiments and is not statistically significant compared to the Doxorubicin positive control groups. "-" indicates that the phenomenon in each column is not observed in the experiments, which may be because the phenomenon is statistically significant compared to the Doxorubicin positive control group or the observation contradicts the hypothesis.

CR1 would show that curcumin decreases cell viability by MTT assay, increases apoptosis by FACS, and decreases phosphor-IkB by western blot. This combination of results fully supports the hypothesis that curcumin is effective. CR2 would show that curcumin decreases cell viability by MTT assay and increases apoptosis by FACS, but curcumin does not decrease phosphor-IkB by western blot. This result partially supports the hypothesis that curcumin is effective. CR3 would show that curcumin decreases cell viability by MTT assay and decreases phosphor-IkB by western blot, but curcumin does not increase apoptosis by FACS. This combination of results partially supports the hypothesis that curcumin is effective. CR4 would show that curcumin increases apoptosis by FACS and decreases phosphor-IkB by western blot, but curcumin does not decrease cell viability by MTT assay. This result partially supports the hypothesis that curcumin is effective. CR5 would show that curcumin decreases cell viability by MTT assay, but curcumin neither increases apoptosis by FACS nor decreases phosphor-IkB by western blot. This result partially supports the hypothesis that curcumin is effective. CR6 would show that curcumin increases apoptosis by FACS, but curcumin neither decreases cell viability by MTT assay nor decreases phosphor-IkB by western blot. This result partially supports the hypothesis that curcumin is effective. CR7 would show that curcumin decreases phosphor-IkB by western blot, but curcumin neither decreases cell viability by MTT assay nor increases apoptosis by FACS. This combination of results partially supports the hypothesis that curcumin is effective. CR8 would show that curcumin does not decrease cell viability by MTT assay, increase apoptosis by FACS, and decrease phosphor-IkB by western blot. This combination of results fully contradicts the hypothesis that curcumin is effective.

6. Concentration of Treatments and Time Duration

Increasing the concentration of curcumin or time duration decreases the viability of MCF-7 cells, induces apoptosis, and decreases the phospho-IkB. Increasing the concentration of curcumin or time duration has no effect on cell proliferation, apoptosis, and the NF-kB signaling pathway. Increasing the concentration of curcumin or time duration has opposite effects. It increases the viability of MCF-7 cells, reduces apoptosis, and increases the phospho-IkB.

7. Discussion

CR1 shows that curcumin decreases viability, increases AnnexinV/PI, and decreases phospho-IkB. This result means that curcumin is effective in reducing proliferation and inducing apoptosis of breast cancer cells. The reduction in cell viability is due to curcumin's cytotoxic effects. The increase of AnnexinV/PI staining indicates that curcumin induces apoptosis, while the decrease in phospho-IkB levels suggests that curcumin inhibits the NF-κB signaling pathway which relates to cell survival and proliferation. This combination of results fully supports the hypothesis that curcumin decreases proliferation and

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induces apoptosis of cells by inhibiting phospho-IkB. Future experiments could involve investigating the detailed mechanisms of apoptosis induction, conducting dose-response studies to determine the optimal concentration of curcumin, and evaluating the impacts of curcumin in combination with other therapeutic agents targeting different signaling pathways.

CR2 shows that curcumin decreases viability and increases AnnexinV/PI, but it does not decrease phospho-IkB. This outcome suggests that curcumin does not suppress the NF-κB signaling pathway but instead decreases cell viability and promotes apoptosis. The cytotoxic and pro-apoptotic effects of curcumin are evident, but the NF-κB pathway might be resistant to curcumin. This combination result partially supports the hypothesis, showing that while curcumin promotes cell death, it does not inhibit NF-κB as expected. The possible reason might be other signaling pathways, such as PI3K/ Akt or MAPK that control cell survival, so the result was not expected. Further experiments could include investigating alternative pathways involved in cell survival, such as the PI3K/ Akt(decrease phospho-Akt) or MAPK(decrease phospho-ERK1/2) pathways, to determine if they compensate for the lack of NF-κB inhibition.

CR3 shows that curcumin decreases viability and decreases phospho-IkB but does not increase AnnexinV/PI. This result suggests that curcumin reduces cell viability and inhibits the NF-κB pathway but does not induce apoptosis. Curcumin cytotoxic effects and NF-κB inhibition are effective, but apoptosis may not be the primary mode of cell death, which may be due to other cell death pathways. This combination result partially supports the hypothesis by showing that curcumin affects cell viability and signaling pathways, but not apoptosis markers. Future experiments could explore other cell death pathways like autophagy, and use additional apoptosis markers (e.g., caspase cleavage, TUNEL assay) to confirm the absence of apoptosis.

CR4 showed that curcumin does not decrease viability, but it increases AnnexinV/PI and decreases phospho-IkB. This outcome indicates that curcumin induces apoptosis and inhibits NF-κB but does not significantly affect cell viability. Apoptotic processes and NF-κB inhibition are activated, but overall cell viability remains unaffected, possibly due to compensatory survival pathways. This combination result partially supports the hypothesis that curcumin decreases proliferation and induces apoptosis of cells by inhibiting phospho-IkB. Future experiments could involve examining compensatory survival pathways, such as autophagy.

CR5 shows that curcumin decreases cell viability, but it does not increase AnnexinV/PI and decrease phospho-IkB. This result suggests that curcumin reduces cell viability but does not induce apoptosis or inhibit the NF-κB pathway. The reason may be that curcumin is not effective to decrease phosphor-IkB and induce apoptosis on MCF-7 cell lines or apoptosis is not the primary mechanism of cell death. This combination result partially supports the hypothesis. Future experiments could include testing different cell lines (MDA-MB-231) to better understand curcumin's effectiveness and investigating non-apoptotic cell death mechanisms, such as necrosis.

CR6 shows that curcumin does not decrease viability nor decrease phospho-IkB, but it induces apoptosis. This outcome suggests that curcumin induces apoptosis but does not reduce viability or inhibit the NF-κB pathway. Apoptosis is observed, but it does not translate to reduced cell viability, possibly due to compensatory survival mechanisms. This combination result partially supports the hypothesis. Future experiments could involve investigating why apoptosis does not affect viability, examining apoptosis markers and cell survival pathways (such as the PI3K/Akt Pathway) in more detail.

CR7 shows that curcumin does not decrease viability nor increase AnnexinV/PI, but it decreases phospho-IkB. This result suggests that curcumin inhibits the NF-κB pathway but does not affect viability or apoptosis. NF-κB inhibition occurs without affecting cell viability or apoptosis, suggesting the pathway inhibition alone is insufficient to reduce viability or induce apoptosis. This combination result partially supports the hypothesis, indicating that NF-κB inhibition is not linked to cell death. Future experiments could include investigating other pathways (such as the PI3K/Akt Pathway) interacting with NF-κB that decrease cell proliferation and increase cell apoptosis.

CR8 shows that curcumin does not decrease viability, increase apoptosis, and decrease phospho-IkB. This result suggests that curcumin has no significant effect on cell viability, apoptosis, or the NF-κB pathway. Curcumin does not cause significant changes in the tested conditions, possibly due to resistance mechanisms or insufficient concentration. This combination result fully contradicts the hypothesis, suggesting that curcumin cannot decrease cell proliferation and cannot increase cell apoptosis. Future experiments could involve re-evaluating experimental conditions (concentration or duration) or testing different cell lines (MDA-MB-231) to test the hypothesis.

8. Increasing Concentration and Time Duration

The concentration gradient of curcumin and time duration have effects on cell proliferation, apoptosis, and the NF-

kB pathway. The result indicates that curcumin significantly reduced phosphor-IkB, increased apoptosis, and decreased cell viability.

The concentration gradient of curcumin and time duration has no effects on cell proliferation, apoptosis, and the NF-kB pathway. A possible explanation might be that curcumin has no effects on proliferation, apoptosis, and NF-kB pathway. Other signaling pathways might control cell survival or cell death. In addition, contamination may also lead to incorrect results. When experiment procedures go wrong, some toxic bacteria might grow in the buffer, which means that these bacterial toxins cause cell death, not curcumin. Therefore, future research can focus on the effects of curcumin on different signaling pathways.

The concentration gradient of curcumin and time duration have opposite effects on cell proliferation, apoptosis, and the NF-kB pathway. It means that curcumin may be useful for other cell lines, or the experimental procedures went wrong, such as contamination on the cells.

9. Conclusion

The purpose of the study is to determine whether curcumin, by inhibiting the NF-kB pathway, has the impact of inhibiting the proliferation and increasing apoptosis in MCF-7 breast cancer cells. MTT assay, FACS, and Western blot will be conducted to test the hypothesis. The result will show whether curcumin has an effect on breast cancer. Future studies could focus on detailed mechanistic research, optimal dosing strategies, and potential combination therapies to enhance curcumin efficacy. Moreover, different cell lines and signaling pathways should be considered during testing the curcumin efficacy.

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