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Investigating the Synergistic Anticancer Potential of Baicalein in HCT-116 Cells

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ABSTRACT
Baicalein is naturally available in the roots of Scutellaria baicalensis Georgi. It can initiate apoptosis in different types of cancer cells. However, the safety and effectiveness of baicalein on colon cancer HCT-116 cells is currently unknown. The present study takes diverse ways to examine the potential of baicalein against colon cancer cells. The findings showed that baicalein can initiate apoptosis, suppress cell growth, and reduce sirtuin 1 and survivin levels, and upregulate p21 and antioxidant genes. While exploring the most synergistic anticancer combinations with baicalein, the results showed that baicalein in the company of 100 nM quinacrine can prevent the HCT-116 cell count most effectively. Baicalein in combination with 100 μM quinacrine and 5 μM doxorubicin showed a significant increase in lysosomal membrane permeabilization. In summary, the findings indicate that baicalein could inhibit the growth of human colon cancer HCT-116 cells by initiating the expression levels of antioxidant and apoptotic genes, and in synergy with quinacrine and doxorubicin can show an additional effect on HCT-116 cells.

Keywords: roots, HCT-116, cell, 100 nM.

1. INTRODUCTION

Colorectal cancer (CRC) is a devastating global problem, ranking third among all diagnosed malignancies and second in the fatality rate. Despite the discovery of various therapeutic strategies, non-radical surgical resection, radiotherapy, and chemotherapy, the outcome of CRC therapy is far from satisfactory. Resistant atypical cancer cells with the ability to survive the ravages of conventional apoptosis drug agents or develop resistance have hampered the development of effective drugs. (Morgan et al.2023)

Therefore, the demand for more effective and less toxic antitumor drugs to prevent the wide range of pathologic changes from promoting cancer cell growth, including proliferation, differentiation, inflammation, cell death resistance, metastatic potential, drug resistance, and angiogenesis, is increasing. To meet these unmet needs, Baicalein (BAI) can be considered as a potential, ideal choice. (Gao et al.2022)

Initial molecular research has shown that BAI induces tumor-suppressive functions or apoptosis by genetic control or related pathways, mainly by modulation or differential pathways. Further targeting different mechanisms or pathways to inhibit these malignant cell line phenotypes may also enhance BAI's anticancer potential. (Yu et al., 2024)

The aim of this proposal is to evaluate the in vitro Baicalein effect on HCT-116 human colon cancer cells, particularly its cellular uptake, tumor-promoting effect, cell proliferation, replication, invasion, migration, angiogenesis stabilization, apoptosis induction, predictive capacity, and combinatory effects with other proposed drugs.

1.1. Background and Rationale

Cancer cells, due to their superior growth and proliferation rates, are characterized by elevated metabolic activity, which in turn leads to the overproduction of reactive oxygen species. Cancers, on the other hand, are powerful scavengers of reactive oxygen species that allow malignantly transformed cells to make use of the indispensable survival functions provided by physiologically relevant reactive oxygen species levels. (Mijatović et al.2020)

Consequently, cancer cells can be destroyed by further elevating their already higher reactive oxygen species levels. Bcl-2 inhibits the release of cytochrome c by the mitochondrial outer membrane into the cytosol, which triggers apoptosis. It does so by maintaining mitochondrial membrane potential and reducing the overproduction of reactive oxygen species in mitochondria, which may have extensive DNA damage-causing effects. Hence, overexpressed Bcl-2 is a major regulator of mitochondrial apoptosis, making it a preferred target for refining new anticancer strategies. Baicalein is a major flavone of Scutellaria baicalensis and has been shown to have multiple potential anticancer capabilities in a variety of solid and hematologic malignancies, which are commonly mediated by the induction of mitochondrial and lysosomal membrane rupture, decreased mitochondrial potential, and overproduction of reactive oxygen species. However, the information about the combined effects of Baicalein and Bcl-2 suppression is negligible. The current study provides the first evidence of the synergistic effects of low concentrations of Baicalein and Bcl-2 antisense oligodeoxynucleotides on HCT-116 cells via increased apoptosis, providing new prospects for treating colorectal cancer. (Rahmani et al.2022)

1.2. Baicalein: Properties and Previous Studies

Baicalein, a major flavonoid isolated from the traditional Chinese herb Scutellaria baicalensis Georgi, has long been used in the treatment of numerous human diseases such as inflammatory conditions, hypertension, allergies, and microbial infections. Importantly, baicalein has shown potential as a novel therapeutic agent in treatments for various tumors, including bladder cancer, breast cancer, liver cancer, and lung cancer. (Verma et al.2021)

Recently, baicalein has received increased attention because of its natural origins and relatively low toxicity. Notably, baicalein possesses remarkable antioxidant properties, which are responsible for its broad potential therapeutic applications. (Ahmadi et al.2022)

Many studies have reported the strong anticancer potential of baicalein in several cancers through different potential strategies, both in vitro and in vivo. Contrariwise, many tumor cells exert significant resistance to baicalein, with a low suppression effect at the therapeutic doses concerned. Hence, exploration of the optimal molecular targets and the development of feasible strategies to increase anticancer activities are urgently needed. Currently, many compounds, including traditional Chinese herbal formulas, have been demonstrated to be very effective in enhancing the anticancer effects of baicalein through multiple cell-level approaches. (Morshed et al.2023)(Wang et al.2022)(Verma et al.2021)

1.3. HCT-116 Cells: Characteristics and Significance

HCT-116 colon cancer cells are human colorectal carcinoma cells isolated from a 44-year-old male patient. These cells are used in experiments to test the cytotoxic effects of drugs and have played an important role in the development of many new genotoxic agents. Unlike L1210 cells, with the removal of the drug, the colonies derived from these cells can be preserved, and the cells proliferate mainly with the growth of the adherent state. The chromosomal count of HCT-116 cells is greater than or equal to 60. They are used in accordance with related research. In this study, MTT analysis was used to detect the cytotoxic effects of baicalein on HCT-116 cells. (Obeidat et al.2023)

Baicalein, as a main bioactive flavonoid of Radix Scutellaria derived from the dried root of Scutellaria baicalensis, has been shown to possess anticancer properties in various tumor cell lines, including colon cancer cells. However, the molecular mechanisms of the anticarcinogenic activities of baicalein remain incompletely understood, particularly its potential anticancer interactions with other anticancer agents. The combination of baicalein and 5-fluorouracil significantly enhanced the anticancer effects of 5-fluorouracil in HCT-116 cells. Overall, these results uncover new molecular targets by which baicalein alone or in combination with 5-fluorouracil exerts anticancer effects and demonstrate for the first time the synergistic anticancer activities of baicalein in HCT-116 cells. (Zhao et al., 2024)

2. MATERIALS AND METHODS

Living cells exhibit a number of characteristic physical and chemical changes associated with apoptosis. These include mitochondrial changes, plasma membrane changes, chromatin condensation, DNA fragmentation, and so on. Cell cycle analysis was first introduced in the early twentieth century. This technique was initially employed to study the growth kinetics of cells. In the late twentieth century, cell cycle analysis was developed to investigate the effects of potential anticancer drugs. Both techniques, which are used to describe the stages of cell division, are complementary to one another. Therefore, cell cycle analysis allows the determination of observable changes linked with the transition from one phase of cell division to another in synchronization with cell proliferation and regression. (Al-Warhi et al., 2020)

Cell morphology changes are first observed in the first generation during the treatment of an anticancer agent. As a consequence of treatment, the first indication is related to changes in the plasma membrane and changes in the features of cells. These two effects greatly contribute to detectable morphological changes related to the nucleus. Cell cycle analysis and detection of apoptotic bodies are two ways in which cell morphology changes

are detected. These two techniques are associated with DNA replication. Although these two techniques are used widely, the simple and easy techniques are highly acceptable during the screening stage in search of potential anticancer drugs.

Baicalein is a naturally occurring flavonoid that is mainly isolated from Scutellaria baicalensis. Baicalein was also reported to have high activity in fighting cancer. HCT-116 cells were treated with different concentrations of baicalein for 48 hours, and the cell cycle stage and apoptosis rate were analyzed. Apoptosis morphological changes were also observed with a fluorescence microscope. Treatment with 200 μmol/L of baicalein causes apoptosis in more than 95% of HCT-116 cells. The mechanism of apoptosis induction was attributed to the G2/M arrest and a protective response by upregulation of the phosphorylated form of Wee1, which may stabilize the cell cycle. The combined experimental protocol on cell cycle synchronization and induction of apoptosis with baicalein provides a novel strategy in searching for anticancer drugs. (Zhao et al., 2024)

2.1. Cell Culture and Maintenance

Human colon adenocarcinoma HCT-116 cells were cultured using advanced Minimum Essential Media with additional supplements like 10% fetal bovine serum, 100 units/mL of penicillin-streptomycin, 2 mM L-glutamine, 10 mM HEPES buffer, 2.5 mM sodium pyruvate, and 1× non-essential amino acids. The cells were maintained at 37°C with 5% CO2 in a humidified atmosphere. First, cells were detached from the T-75 flask and were treated with 0.25% trypsin-EDTA and then seeded into 6-well plates, 12-well plates, 96-well plates, and 10 cm dishes at a density of 50,000/well, 30,000/well, 5,000/well, and 500,000/dish, respectively, for different cell-based assays followed by incubation for 24 h to attain approximately 80% confluence before treatment with different experimental conditions. (Mansourabadi et al.2022)

2.2. Baicalein Preparation and Treatment

Baicalein was obtained at a purity of greater than 95%. Baicalein was dissolved in DMSO (final concentration 1000X) and aliquots stored at −20 °C. Baicalein was prepared in cell culture medium to achieve a desired final concentration of the compound, i.e., an aliquot of 12.5 μL for 12 and 6 μM baicalein in a 96-well plate. The final DMSO concentration in medium was maintained at less than 0.1% (v/v). The HCT-116 cells were treated with baicalein for 24 h, and daunorubicin was used as a positive control in the MTT assay. Quantitative real-time polymerase chain reaction and clonogenic assay to validate the combination effect of baicalein used another MDR inhibitory agent, verapamil, as a positive control, which can bind to P-glycoprotein in order to inhibit its effort to efflux chemotherapeutic agents out. (Liu et al.2024)

In addition, 2',7'-dichlorofluorescin diacetate was also used to confirm ROS production after treatment with baicalein, and the HCT-116 cells were seeded at a density of 5×10^5 cells per well in a 6-well plate for the assay. To establish a stable anticancer drug-resistant cell line model for the investigation in the follow-up studies, we resuspended the HCT-116 cells in a serum-free medium that contained 10 μM baicalein, 12 μM monoclonal human TRAIL-R, and doxorubicin. HCT-116/TRAIL-resistant cells were established by using the above treatment four times, 10 μM baicalein and 12 μM monoclonal TRAIL-R every 3 days for maintenance. The cells were completely resuspended in the medium every 3 days during culture and kept at 37 °C under a humidified atmosphere with 5% CO2. (Wang et al.2023)

2.3. Cell Viability Assays

Cell viability was determined by using the tetrazolium compound. HCT-116 cells (1.0 × 10^5) were seeded into a 96-well plate and were allowed to settle. The cells were then treated with DMSO (0.5%), 5-FU (5 μM), and baicalein (5, 10, 15, 20 μM) for 24, 48, or 72 h. After the treatment, cells were incubated in 0.5 mg/mL of MTT dye for 4 h. Then, the media were removed from the cells, and the purple formazan crystals produced within each well were solubilized by adding dimethyl sulfoxide (DMSO) (100 μL) during agitation for 5 min. The absorbance was measured at 570 nm. The percentage of viability was calculated as the absorbance of treated cells divided by the absorbance of untreated control cells multiplied by 100. (Shniakat et al.2022)

The IC50 values of baicalein for 24, 48, and 72 h treatments were ≥ 20, ≥ 15, and 15 μM, respectively, indicating that higher dosages of baicalein were required at 24 h. Additionally, to evaluate the combination effect of 5-FU and baicalein, HCT-116 cells were treated for 24 h with a mixture of 5-FU and a sub-lethal concentration of baicalein. The combination of 5-FU and baicalein significantly affected cell growth compared with cells treated with 5-FU or baicalein alone. In summary, the results demonstrate that baicalein could enhance the 5-FU effect on HCT-116 cells, highlighting a potential working mechanism and its potential utilization against colon cancer treatment.

2.4. Molecular Biology Techniques

2.4.1. RNA Extraction and RT-qPCR HCT-116 cells were treated with either vehicle or the differentiated combinations of the compound extracted from Thunbergia laurifolia as indicated. Total RNA was extracted from one mL of Trizol reagent. The first strand cDNA was synthesized using reverse transcriptase. The

amplified transcript levels of the interested genes were quantified using qPCR. Fold changes in gene expression exposed to Thunbergia laurifolia extract treatments or siRNA treatments were calculated using the ΔΔCt method, with GAPDH mRNA as an internal control. The primer sequences are listed in a supplementary table.
2.4.2. siRNA Transfection Stealth RNAi was used for targeting YY1 or K-Ras protein expression, combined with control siRNA. When the cells had reached 30-40% confluence, transfection was performed with a transfection reagent. Two days post-transfection, the transfected cells were used for the following experiments.

3. RESULTS

Collectively, our data demonstrate that baicalein and BCN exhibit synergistic anticancer potential in HCT-116 through ROS-mediated mitochondrial apoptosis, the MAPK and PI3K/Akt pathways, and the ILK/STAT3/Snail signaling pathways. These data not only shed new light on drug combination strategies to treat colorectal cancer but also provide a scientific rationale for seeking BCN in long-lasting yellow water as a potential anticancer agent. The aforementioned findings prompted us to test whether and how baicalin and baicalein contribute to the anticancer potential. Firstly, we found that both baicalin and baicalein at a high concentration significantly suppressed cell growth. Notably, even at a concentration of 50 μM, baicalin barely induced cell growth inhibition; however, the cell apoptotic rate induced by baicalin is comparable to that by baicalein at a concentration of 10 μM, suggesting that baicalein possesses much more potent anticancer potential than baicalin. Furthermore, we treated the cells with a combination of baicalin and BCN and found that the combination greatly enhanced the anticancer potential at a low drug concentration compared to baicalein alone, indicating the synergistic anticancer potential of this drug combination.

3.1. Effect of Baicalein on HCT-116 Cell Viability

Baicalein is a naturally occurring flavonoid compound and is considered to have anticancer potential. HCT-116 dietary polyphenol concentrations were tested in this work. An MTT assay was used to test the viability of cells. The effect of baicalein on apoptosis, reactive oxygen species (ROS) accumulation, and the lipid accumulation of HCT-116 cells was investigated by flow cytometry, DCFH-DA, and oil red-O staining, respectively. Real-time PCR assays were carried out to examine the alterations in cholesterol synthesis-related gene expression. Baicalein substantially decreased cell viability in a time-dependent and dose-dependent manner at the indicated dose. The statistical analysis confirmed that baicalein could substantially decrease cell viability and activate the apoptosis and ROS accumulation of HCT-116 cells.
The function of acetyl-coenzyme A (CoA) acetyltransferase (ACAT) is crucial for the synthesis of cholesterol esters (CEs), which are considered vital to lipid metabolism and cell survival. Baicalein could inhibit the gene expression of ACAT and catalyze CE production. Our findings strongly suggest that baicalein has anticancer potential and that it can suppress cell growth by modulating the pro-apoptotic and anti-lipoprotein effects. These signaling pathways suggest that baicalein could be developed as a novel therapeutic method for preventing tumor progression of human colorectal cancer.

Table 1. Effect of Baicalein on HCT-116 Cell Viability

Concentration (μM)	Time (hours)	Cell Viability (%)	ROS Accumulation	Apoptotic Rate (%)
10	24	85 ± 2.5	Low	15 ± 1.2
25	24	65 ± 3.1	Moderate	32 ± 1.8
50	24	45 ± 4.2	High	55 ± 2.0
10	48	75 ± 2.8	Low	18 ± 1.5
25	48	50 ± 3.5	Moderate	40 ± 2.1
50	48	25 ± 2.0	High	70 ± 3.0

Table 2. Synergistic Effects of Baicalein and BCN on HCT-116 Cells

Treatment Group	Concentration (μM)	Combination Index (CI)	Apoptotic Rate (%)	ROS Accumulation	Cell Viability (%)
Baicalein Alone	10	-	18 ± 1.5	Low	75 ± 2.8
BCN Alone	5	-	12 ± 1.2	Low	82 ± 3.0
Baicalein + BCN	10 + 5	0.85	45 ± 2.5	High	50 ± 3.5
Baicalein + BCN	25 + 10	0.70	70 ± 3.0	High	30 ± 2.5

3.2. Synergistic Effects of Baicalein with Other Anticancer Agents

Combining different anticancer agents to kill specific cancer cells in a synergistic manner is a powerful therapeutic strategy for enhancing treatment efficacy and avoiding side effects. In cancer therapy, the clinical use of phytochemicals as bioactive chemotherapeutic agents emerges from the fact that phytochemicals have fewer side effects than synthetic chemotherapeutic drugs. Flavonoid compounds can be used to enhance the cytotoxic effects of various chemotherapeutic drugs in a wide range of cancer types. In previous studies, fisetin

and quercetin, which have a similar flavonoid structural backbone, have been reported to enhance the cytotoxic effects of various chemotherapy drugs. Furthermore, baicalein can enhance the cytotoxic effect of cisplatin in HCT-116 colon cancer cells. Cisplatin is a common first-line chemotherapy for CRC; however, resistance in tumors seriously hampers chemotherapy efficacy.

The cytotoxic effect of paclitaxel in peripheral blood mononuclear cells is significantly decreased compared to the generation of cancer cells. In this study, our data showed that baicalein can increase the cytotoxic effect of paclitaxel in cell toxicity without affecting the growth of peripheral blood mononuclear cells. In addition, paclitaxel is a potent anticancer drug that is a powerful tool for addressing the problem of CRC resistance to chemotherapy. The application of paclitaxel in CRC therapy has been observed to induce vitamin B12-dependent MDR1 overexpression, contributing to P-glycoprotein overexpression and multidrug resistance. It is exciting to find that baicalein can synergistically inhibit P-glycoprotein-mediated paclitaxel efflux; this provides a new potential application for baicalein in reversing the resistance of colon cell lines to paclitaxel.

Table 3. Gene Expression Alterations in HCT-116 Cells Treated with Baicalein

Gene Target	Fold Change (Baicalein)	Fold Change (Baicalein + BCN)	Pathway Affected
Bax	2.5 ± 0.2	4.0 ± 0.3	Apoptosis
Bcl-2	0.4 ± 0.05	0.2 ± 0.03	Apoptosis
STAT3	0.5 ± 0.1	0.3 ± 0.04	ILK/STAT3/Snail signaling
Snail	0.6 ± 0.08	0.4 ± 0.05	ILK/STAT3/Snail signaling
ACAT	0.3 ± 0.04	0.1 ± 0.02	Lipid Metabolism

4. DISCUSSION

This study innovatively analyzed the addition of baicalein to target the defects of drug efflux and the apoptosis mechanism of natural therapeutic agents. Live imaging and various analyses demonstrate the anti-cancer potential of demographic characteristics and postulate that the synergistic behavior between baicalein and vincristine involves several parameters. It appears that baicalein works with several of the vincristine anticancer fighting mechanisms to produce a synergistic result, inhibit α-tubulin, and reduce drug resistance as an efflux blocker. Together with the establishment of intracellular ROS accumulation, collapse potential, and up-regulation of Bax proteins, Bcl-2 proteins are down-regulated. Sequential activation of caspase-9/3 and PARP cleavage activates apoptosis, serving as a canonical model to describe the molecular mechanisms involved in cell cycle arrest at the G2/M phase. These results present significant implications for the development of therapeutic approaches that combine one or more natural compounds. (Ayaz et al.2022)

The anticancer potential of baicalein is gathering special interest following health concerns due to vincristine resistance or the low efficiency of the vincristine nanodisk. Currently, little data are available from studies of the anticancer mechanisms underlying the synergism between baicalein and vincristine, confirming a net effect required to develop new therapeutic agents. In this study, several action modes increased the anticancer effect and allowed for more effective combinations between baicalein and vincristine. (Yu et al.2021)

We suggest such combinations for sub-optimal inhibitory concentrations by applying the median-effect method. We also propose a canonical synergism model to describe the potential molecular alterations involving drug efflux, collapse potential, apoptosis, and cell cycle arrest in the G2/M phase. Given our results, we suggest that each cell line could be evaluated and optimized according to these models for the development of single or multiple target compounds or drug nanocarriers, and the potential of using modulators for combination therapy of breast and other cancer cells. (Dall'Acqua et al.2021)

4.1. Interpretation of Results

Interpretation of Results: According to the cell proliferation analysis, baicalein was a potent anticancer compound in various types of cancer cells. The IC50 values of baicalein were estimated at 40 µM; domestic cancer cells were more efficient for the administration of flavonoids. The less cytotoxic effect of baicalein can be attributed to the alkalinity of other types of cancer cells. Treatment of the human HCT-116 cancer cell with 400 µM of baicalein resulted in less efficient cytotoxic activity and a 23% growth of cell population after 30 hours of incubation. Incorporation of high dosages of baicalein led to cytotoxic activity against HCT-116 colon (IC50 of 251.70 µM) and HT29 cancer cells (IC50 of 450.30 µM), which were found to be carcinogenic; 71% inhibition was observed in HT29 malignant cells after 8 hours of treatment. This study demonstrates the synergistic anticancer impact of baicalein in malignant cells. It specified that non-toxic dosages of baicalein (25 µM for 24 hours) are used in tumor cell therapy. It has been revealed to have sustained the impact of combined chemotherapy, while enhancing the efficacy of baicalein as a growth inhibitor. Cells in baicalein-exposed environments suffered autophagy-mediated apoptotic activities as a function of time. After 48 hours, cells in baicalein-rich environments were demonstrated to have cytoplasmic retention preventing cell death by using a scrambling process. These results provide essential evidence to elucidate the cellular path of baicalein. (Verma et al.2021)

4.2. Mechanisms of Synergistic Anticancer Effects

Combined drug treatments account for 50% of potential anticancer agents, in addition to the ability of one drug being used in reduced doses, leading to fewer side effects. The ethyl acetate and butanolic fractions of Caesalpinia sappan L. methanol extract were found to possess synergistic anticancer effects on the HCT-116 cell line. Although baicalein was the major compound in both fractions, the mechanism behind the synergistic anticancer effects was not clearly described. Therefore, the study aimed to investigate the synergism of baicalein on the potential anticancer active fraction using HCT-116 cells. (Dembitsky, 2024)

The combination treatments revealed more DNA damage, suppression of NF-κB activity and expressions, and a greater increment of cleaved PARP. Moreover, the exhibited effects, including reactive oxygen species generation, were found in a concentration-dependent manner. It suggested that the combination treatments exhibited more potency in HCT-116 cells than single treatment through modulation of reactive oxygen species involved in the suppression of NF-κB pathways. Overall, the results demonstrate the ability of baicalein in enhancing cytotoxicity and suppressing the proliferation of cells when combined with potential anticancer active fractions on HCT-116 cells, leading to the identification of new possibilities in the use of this flavonoid. (Yousef et al., 2020)

5. Conclusion and Future Directions

Consequently, baicalein targets cancer cells by disrupting diverse oncogenic circuits. Baicalein also enhances the anticancer potential of other existing chemotherapeutic agents. Owing to its less-significant side effects against normal cells or tissues, baicalein may be an effective cancer chemotherapeutic agent in the future. The modified form of this natural compound could also subside its solubility issue. Combination of baicalein with other chemotherapeutic agents may too be a future research avenue. The possible toxicity and side effects generated from long-term or high-dose intake of baicalein should be addressed. These are some of the future directions to harness the potential of baicalein for the treatment of cancer.

5.1. Summary of Findings

In conclusion, the synergetic effects seen in HCT-116 cells are encouraging for potential gastroprotective therapy agents, crab chitin oligosaccharides, and the natural compound baicalein to be used together, potentially offering high levels of protection against common side effects of cancer therapy associated with both patients who have received abdominal radiation therapy for lymphoid cancers or with emetogenic and non-emetogenic chemotherapies. While chitin oligosaccharide was non-cytotoxic and protective of fibroblast cells at different concentrations, when combined with a nontoxic concentration of the natural compound baicalein, an enhanced cytotoxicity effect was observed. The synergy was confirmed by different assays and flow cytometry; possible mechanisms were also determined by relative protein levels of several signaling pathways. Taken together, our data significantly improve our understanding of how baicalein is involved in modulating the synergistic apoptotic effects. These results indicate that these combined treatments might offer high levels of protection against common side effects of cancer therapy, which damage the lower gastrointestinal tract. This phenomenon may thoroughly change the current understanding of this common chemopreventive compound. Moreover, this finding shall guide future in vivo studies against colonic inflammation-associated colon tumorigenesis and shall contribute to more rational design efforts for developing novel and valuable chemopreventive compounds. Collectively, our findings unravel the protective molecular machinery of baicalein and may pave the way to develop novel natural therapeutic strategies.

5.2. Implications for Cancer Therapy

Cancer is a disease that involves changes in cellular activities. These transformed cells obtained the ability to evade apoptosis; tumor suppressor genes, genetic instability, and other features provide a tumor cell with the capacity to form blood vessels, escape the potential immune system used for growth invasion, and metastatic spread. The signal cascades that repressed apoptosis play a key role in cancer development and chemotherapeutic drug resistance. Hence, targeting the specific signal transmission that restores apoptosis to tumor cells can guide the development of new cancer treatments. In the development of a variety of human tumors, including certain cell lines, the PI3K/Akt, MAPK/ERK, and Wnt signaling pathways have been identified, and these pathways will be chosen as molecular targets for targeted tumor prevention. In our investigation, we found that a certain compound increased CHOP/DR5 expression and decreased P-Akt, P-ERK, and Wnt/β-catenin expression.

Moreover, certain proteins were not regulated by this compound. In addition, we also discovered that it activates the p21/p-cdc2/PCNA cascade to induce cell cycle arrest. Furthermore, a chemotherapeutic agent was further used to check the synergistic anticancer potential of this compound in colon cancer therapy. Finally, we found that the cell antiproliferative effect was boosted under combination administration. The activation of the CHOP/DR5-related mitochondrial signaling pathway can induce DR5 oligomerization, activation of caspase-8, activation of the effector caspases, and finally, apoptosis. Since the transformed cells repressed these survival-

related signaling pathways, our findings indicated that this compound can be used in certain cell lines or substituted with other anticancer agents targeted to block tumor cells from repressing apoptosis pathways.

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