Cellular and Molecular Biology
E-ISSN : 1165-158X / P-ISSN : 0145-5680
www.cellmolbiol.org

Effects of curcumin and soy isoflavones on genomic instability of human colon cells NCM460 and SW620

Cao Yu[1,2], Lu Jianying[1], Wang Han, Wang Xu[1,3*], Ni Juan[1,*]

[1] School of Life Sciences, The Engineering Research Center of Sustainable Development and Utilization of Biomass Energy, Yunnan Normal University, Kunming 650500, China
[2] Yunnan Tumor Research Institute, The Third Affiliated Hospital of Kunming Medical University, Kunming 650118, China
[3] Yeda Institute of Gene and Cell Therapy, Taizhou, 318000, China

ARTICLE INFO

Original paper

Article history:
Received: November 15, 2022
Accepted: January 20, 2023
Published: January 31, 2023

Keywords:

Curcumin, soy isoflavones, colorectal cancer, genomic instability, apoptosis

ABSTRACT

Curcumin (CUR) and soy isoflavones (SIs) are two plant-based polyphenols that have attracted much attention, because of their extensive anticancer and health maintenance effects. However, the relevant molecular mechanisms are still uncertain. Genomic instability (GIN) refers to a combination of gene abnormal amplification, sequence deletion, ectopic, and other types of gene damage in cells, and it is one of the main factors causing cells to lose normal physiological functions. Therefore, we used the cytokinesis-block micronucleus cytome (CBMN-Cyt) assay as the main research method to analyze the effects of CUR and SIs on the GIN of human normal colon cells NCM460 and colon cancer cells SW620. Results show that CUR (12.5 μM) could reduce the apoptosis of NCM460 and maintain its genomic stability while inhibiting the proliferation of SW620 and promoting its apoptosis. There was no difference in the promoting effect of GIN between SW620 and NCM460 using SIs (3.125–50 μM). When the two polyphenols (*v*/*v* = 1/1, 1.5625–6.25 μM) were mixed, they could promote the proliferation and GIN of the NCM460 and SW620 cells, but we did not find that combining the two produced a better effect on the cells. In conclusion, CUR has more prominent health and anticancer effects, and it may become a dietary recommendation for daily health maintenance and a potential adjuvant drug for cancer treatment.

Doi: http://dx.doi.org/10.14715/cmb/2022.69.1.7
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Introduction

Phytochemicals are bioactive non-nutrient compounds widely distributed in plants with an ability to reduce the risk of various diseases (1, 2). Polyphenolic compounds are phytochemicals, generally discovered in plants, which are synthesized to adapt to stressful and pathogenic environmental situations (3). Therefore, they possess many health-promoting properties, mainly antioxidant, anti-inflammatory, and antibacterial. Recently, there has been increasing interest in polyphenols in the treatment of different pathological states, such as cardiovascular and neurodegenerative diseases and cancer (4-6). Many researchers are enthusiastic about the anticancer properties of polyphenols (7-9). Polyphenols exert their potential in anticancer therapy by promoting apoptosis and cell senescence, regulating autophagy, and inhibiting the proliferation and migration of cancer cells.

Curcumin (CUR) is a diketone compound extracted from the rhizomes of some plants in the Zingiberaceae and Araceae families. It is a rare pigment with a diketone structure in the plant kingdom, and it is a natural dietary polyphenol with a slightly bitter taste and insolubility in water. CUR is mostly used as a colorant and additive in food production. It has strong antioxidative, anti-inflammatory, antimicrobial (10-14), and broad-spectrum antitumor pharmacological activities (15). Specifically, the strong antitumor activity and organ-protective effect (16, 17) make CUR an attractive anticancer active ingredient in traditional Chinese medicine.

Soy isoflavone (SIs) are secondary metabolites formed during soybean growth, which are biologically active substances. Because they are extracted from plants and have a similar structure to estrogen, SIs are also called phytoestrogens. The estrogenic effect of SIs influences hormone secretion, metabolic biological activity, protein synthesis, and growth factor activity. They can effectively improve the symptoms of osteoporosis (18), type 2 diabetes (19), hypercholesterolemia, and cardiovascular disease (20, 21), along with a certain antitumor activity (22). In Asian countries, where soybean is one of the most popular crops, the recommended intake is 20–50 g per day (23, 24).

Genomic instability (GIN) is the basic cause of many human genetic–environmental diseases, and it is highly correlated with the increased risk of birth defects, immunodeficiencies, and degenerative diseases, such as Alzheimer's disease, cardiovascular disease, and tumors. A previous study by our group found that polyphenols may induce high levels of GIN, thereby causing cancer cells to undergo apoptosis, such as resveratrol, tea polyphenols, and geranium. This may represent one of the mechanisms underlying their anticancer activity (25-27). Can CUR

* Corresponding author. Email: wangxu@fudan.edu.cn (X.W.); gt_gg30@163.com (J.N.)

and SIs also inhibit colon cancer cell growth by altering GIN, and can the combination of the two substances exert a better tumor suppressor effect? This study aimed to use human colonic epithelial cells NCM460 and human colon cancer cells SW620 as the research objects to analyze the effects of the two active polyphenols on GIN and proliferation in normal and cancer cells, in addition to providing a theoretical basis for their application in anticancer therapy and health maintenance.

Materials and Methods

Chemicals

CUR (purity ≥ 98%) was obtained from Yuanye Bio-Technology (Shanghai, China), SIs (HPLC ≥ 85%) were obtained from Solarbio® life sciences (Beijing, China), and cytochalasin-B was obtained from Sigma Aldrich (St. Louis, MO, USA). Stock solutions of CUR (54.3 mM), SIs (76.5 mM), and cytochalasin-B (600 μg/mL) were prepared in dimethyl sulfoxide (DMSO). The solution was stored at −20 °C and diluted to the desired concentration in a medium immediately before use. The final concentration of DMSO never exceeded 0.25% (*v*/*v*); this concentration was found to not exert any cytotoxic or genotoxic effects (25).

Cell culture

NCM460 and SW620 are both adherent cell lines; they were obtained from the Kunming Institute of Zoology, CAS (Yunnan, China). They were maintained as a monolayer in 75 cm^2 flasks (Corning, NY, USA) in RPMI-1640 medium (Gibco, NY, USA) supplemented with 10% newborn calf serum (Gibco, NY, USA), 1% penicillin (5000 IU/mL)/streptomycin (5 mg/mL) solution (Gibco, NY, USA), and 1% L-glutamine (2 mM) (Gibco, NY, USA), and they were kept at 37 °C in a 5% CO_2 environment. In order to ensure that endogenous GIN did not occur significantly, both cells were used at early passages (ranging from P15 to P25) for this study.

Trypan blue exclusion assay

NCM460 and SW620 cells were both seeded into 24-well plates (Corning, NY, USA) at a density of 1×10^5 cells/mL and exposed to different concentrations of CUR or SIs (0, 1.5625, 3.125, 6.25, 12.5, 25, 50, and 100 μM). After 24 h incubation, adherent and nonadherent cells were detached from plates and collected. Cells were incubated with trypan blue to exclude dead cells and then counted using a hemocytometer. This procedure was repeated three times in duplicate for each CUR or SI concentration.

MTT assay

NCM460 and SW620 cells were both seeded into 96-well plates (Corning, NY, USA) at a density of 2.5×10^4 cells/mL and exposed to different concentrations of CUR and SIs (0, 3.125, 6.25, 12.5, and 25 μM). After 24 h incubation, 10 μL of 5 mM MTT was added to each well. Cells were incubated with MTT at 37 °C for 4 h. Then, 100 μL of DMSO was added to each well to replace MTT. After incubating at 37 °C for 10 min, we used a microplate reader to detect the absorbance of each well at 570 nm. This procedure was repeated three times in duplicate for each concentration.

Cytokinesis-Block Micronucleus Cytome (CBMN-Cyt) assay

The CBMN-Cyt assay was performed as previously described (28). In brief, NCM460 or SW620 cells were seeded into 24-well plates at a density of 1×10^5 cells/mL and cultured in RPMI-1640 medium containing CUR (0 and 12.5 μM), SIs (0, 3.125, 12.5, and 50 μM), or CUR and SIs (0, 1.5625, 3.125, and 6.25 μM) for 24 h. The medium was discarded after treatment, and cells were washed twice with phosphate-buffered saline (PBS, pH 7.2). Fresh medium with 1.5 μg/mL cytochalasin B was added to each culture to block cytokinesis and rinsed with PBS after a further 24 h. Cells were centrifuged onto glass slides using a cyto-centrifuge for 5 min at 800 rpm (100× *g*). The final cell density per slide was maintained between 0.5×10^5 and 1×10^5 cells. After drying briefly in air, slides were fixed in 100% cold methanol at −20 °C for 15 min and stained with 10% Giemsa (San'ersi, Shanghai, China). The slides were washed twice in ddH_2O and then allowed to air-dry on a cover slip. Stained slides were encoded to ensure a blind microscopic analysis, and the code was not removed until the end of the microscopic analysis. All biomarkers of CBMN-Cyt were scored under 1000× magnification using an optical microscope (Olympus, Tokyo, Japan) by one person, according to Michael's (29) criteria. A total of 1000 binucleated cells (BNCs) were scored per group to determine the frequency of micronuclei (MNs), nucleoplasmic bridges (NPBs), and nuclear buds (NBUDs).

Nuclear Divided Index (NDI) and cell apoptosis

After mounting slides as described for the CBMN-Cyt assay, the number of mononuclear, binucleate, trinucleate, and multinucleated cells in 500 cells for each group was microscopically counted, and NDI was calculated. Apoptotic cells were counted according to the criteria described by Michael (29), with at least 500 cells per group.

Statistical analysis

All statistical analyses were performed using SPSS 22.0 for windows (SPSS, Chicago, IL, USA). The Kolmogorov–Smirnov test was used to test the normality of all datasets. The differences in observed values among the control and polyphenol-treated groups were analyzed using an independent-sample *t*-test or one-way analysis of variance (ANOVA). First, Levene's test was performed to examine the homogeneity of variances among the control and polyphenol-treated groups. Second, post hoc tests (Tukey's test was used when the equality of variance assumption held ($p > 0.05$), while the Dunnett T3 test was used otherwise ($p < 0.05$)) followed in case a significant effect was detected. We only considered differences with a *p*-value (two-tailed) lower than 0.05 as significant. All the figures were graphed using GraphPad PRISM 5.0 (GraphPad, San Diego, CA, USA). Of note, we used 0 μM polyphenols to represent the vehicle-only controls (CUR: 0.185% DMSO, SI: 0.131% DMSO, CUR and SI: 0.024% DMSO, *v*/*v*) in all figures.

Results

The inhibitory effect of CUR or SIs on SW620 cell viability

The trypan blue exclusion assay revealed that some concentrations (CUR: 6.25, 25, 50, and 100 μM, $p < 0.01$,

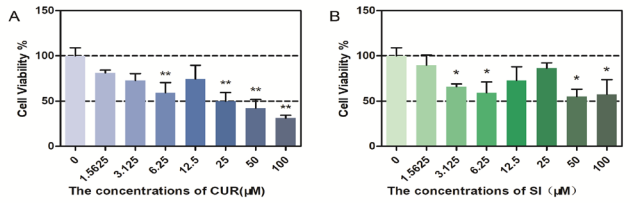

Figure 1. Influence of CUR (A) or SI (B) treatment on the viability of SW620 cells for 24 h. SW620 cells with a seeding density of 1 × 10^5/mL were incubated without or with CUR/SIs (0.01–100 μM) for 24 h; then, cell viability was determined using the trypan blue exclusion assay. * $p < 0.05$; ** $p < 0.01$ vs. untreated control.

Figure 1A; SIs: 3.125, 6.25, 50, and 100 μM, $p < 0.05$, Figure 1B) resulted in a significant decrease in SW620 cell viability. According to the above experimental results, we selected CUR (12.5 μM) and SIs (3.125, 12.5, and 50 μM) for the CBMN-Cyt assay to explore the effects of polyphenol treatment on cell GIN, NDI, and apoptosis.

CUR had different effects on GIN in SW620 and NCM460 cells

By counting and analyzing the results of the CBMN-Cyt assay, we found that 12.5 μM CUR could significantly increase GIN ($p < 0.05$, Figure 2A), reduce NDI ($p < 0.01$, Figure 2C), and induce cell apoptosis ($p < 0.05$, Figure 2D). One of the main changes in GIN was an increase in NPB ($p < 0.05$, Figure 2B).

Subsequently, we determined the changes induced by CUR in NCM460 cells. By counting and analyzing the results of the CBMN-Cyt assay, we found that 12.5 μM CUR could significantly decrease the GIN ($p < 0.01$, Figure 3A) and reduce cell apoptosis ($p < 0.01$, Figure 3D). The changes in GIN affected all three indicators ($p < 0.01$, Figure 3B).

CUR treatment for 24 h had a good inhibitory effect on the proliferation of SW620 cells, while it could also effectively reduce the GIN and maintain the proliferation state of NCM460 cells. We then aimed to determine if this potentially beneficial effect on normal colonic epithelial cells NCM460 continued after 24 h of treatment. Accordingly, we continued to culture the NCM460 cells after 24 h of CUR treatment for 9 days. The CBMN-Cyt assay revealed that, after 9 days, CUR was still able to reduce GIN ($p < 0.01$, Figure 4A) and inhibit the apoptosis ($p < 0.05$, Figure 4D) of NCM460 cells. The improvement in GIN was mainly reflected by the reduction in MN and NBUDs ($p < 0.05$, Figure 4B). Compared with 24 h, there was a weakened reduction in GIN, but the effect remained. Usually, when we verify whether a drug has a good anticancer effect, we mainly focus on whether it persistently inhibits the growth of cancer cells. Therefore, in this experiment, NCM460 and SW620 cells were applied in the same short-term acute treatment to observe the effect of GIN. Then, the NCM460 cell evaluation was continued for 9 days to detect any potential negative impact of the drug residue on normal cells. Experimental results revealed that the metabolic retention of CUR still had no potential negative effects on normal cells.

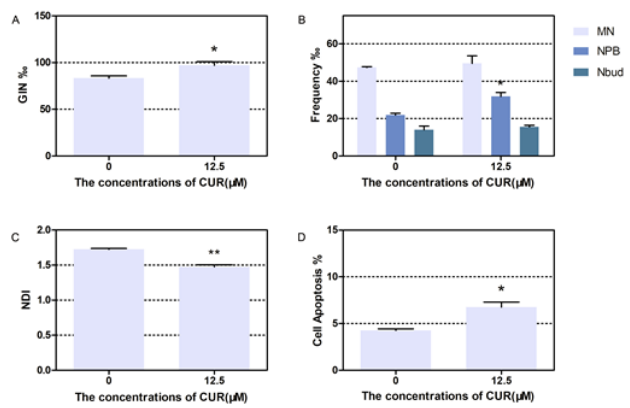

Figure 2. Influence of CUR treatment on the GIN, NDI, and apoptosis of SW620 cells for 24 h. SW620 cells with a seeding density of 1 × 10^5/mL were incubated without or with CUR (12.5 μM) for 24 h; then, GIN (A), cell indicators (B), NDI (C), and apoptosis (D) were evaluated using the CBMN-Cyt assay; * $p < 0.05$, ** $p < 0.01$ vs. untreated control.

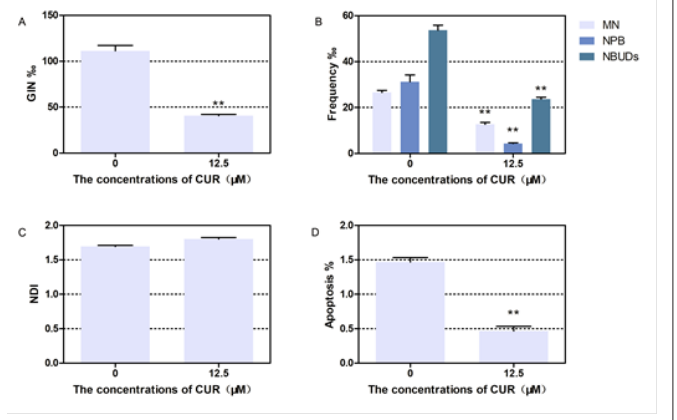

Figure 3. Influence of CUR treatment on the GIN, NDI, and apoptosis of NCM460 cells for 24 h. NCM460 cells with a seeding density of 1 × 10^5/mL were incubated without or with CUR (12.5 μM) for 24 h; then, GIN (A), cell indicators (B), NDI (C), and apoptosis (D) were determined using the CBMN-Cyt assay; * $p < 0.05$, ** $p < 0.01$ vs. untreated control.

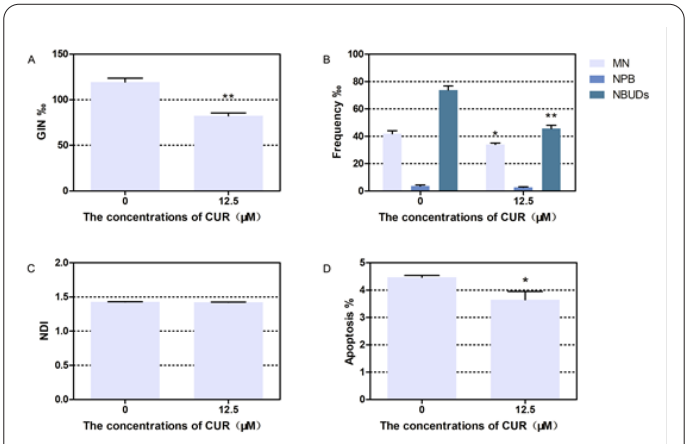

Figure 4. Influence of CUR treatment for 9 days on the GIN, NDI, and apoptosis of NCM460 cells. NCM460 cells with a seeding density of 1 × 10^5/mL were incubated without or with CUR (12.5 μM) for 24 h and then continued culturing for 9 days. GIN (A), cell indicators (B), NDI (C), and apoptosis (D) were determined using the CBMN-Cyt assay; * $p < 0.05$, ** $p < 0.01$ vs. untreated control.

Effect of SIs on GIN in SW620 and NCM460 cells

Using the CBMN-Cyt assay, we found that SIs at

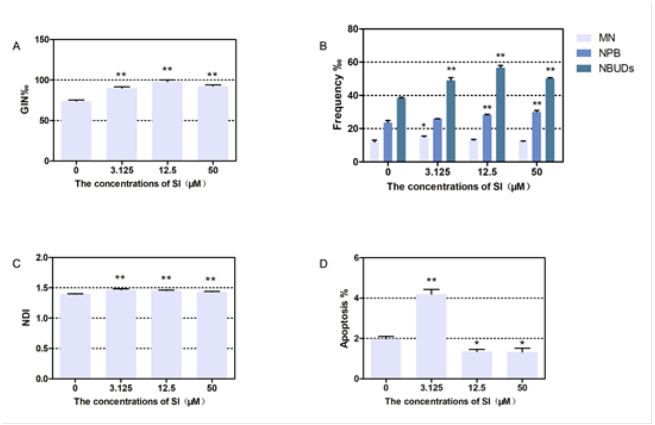

Figure 5. Influence of SI treatment on the GIN, NDI, and apoptosis of SW620 cells for 24 h. SW620 cells with a seeding density of 1 × 10^5/mL were incubated without or with SIs (3.125, 6.25, or 12.5 μM) for 24 h; then, GIN (A), cell indicators (B), NDI (C), and apoptosis (D) were determined using the CBMN-Cyt assay; * $p < 0.05$, ** $p < 0.01$ vs. untreated control.

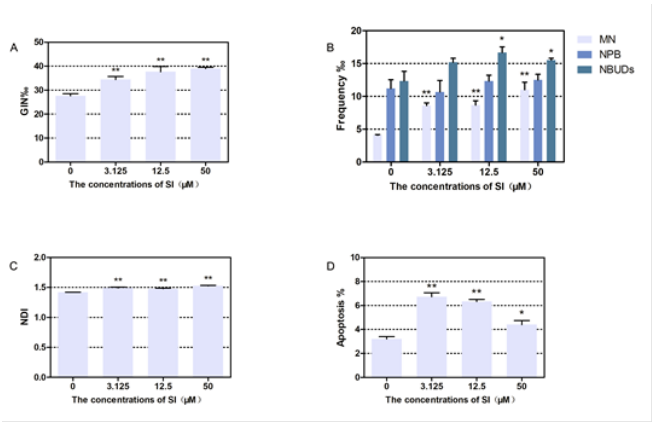

Figure 6. Influence of SI treatment on the GIN, NDI, and apoptosis of NCM460 cells for 24 h. NCM460 cells with a seeding density of 1 × 10^5/mL were incubated without or with CUR (12.5 μM) for 24 h and then continued culturing for 9 days. GIN (A), cell indicators (B), NDI (C), and apoptosis (D) were determined using the CBMN-Cyt assay; * $p < 0.05$, ** $p < 0.01$ vs. untreated control.

3.125–50 μM could significantly increase GIN ($p < 0.01$, Figure 5A), with the increase in NBUDs as the main indicator ($p < 0.01$, Figure 5B). MN showed a slight increase at 3.125 μM ($p < 0.05$, Figure 5B). Furthermore, there was a slight increase in NPB at 12.5 and 50 μM ($p < 0.01$, Figure 5B). However, SIs at 3.125–50 μM could significantly increase NDI ($p < 0.01$, Figure 5C). Whereas SIs at 3.125 μM could significantly promote SW620 apoptosis ($p < 0.01$, Figure 5D), SIs at 12.5 and 50 μM inhibited cell apoptosis ($p < 0.05$, Figure 5D), suggesting that the inhibitory mechanism of SIs toward SW620 cell proliferation is more complicated and does not completely depend on the change in GIN. Furthermore, the results indicated a better anticancer effect of SIs at a lower concentration (i.e., 3.125 μM).

Moreover, we verified the effects of SIs on GIN, NDI, and apoptosis in NCM460 cells. After 24 h of SI treatment, we found that 3.125–50 μM concentrations could increase the GIN of NCM460 cells ($p < 0.01$, Figure 6A), increase NDI ($p < 0.01$, Figure 6C), and promote the occurrence of apoptosis ($p < 0.05$, Figure 6D). Specifically, the change in GIN was mainly based on the increase in MN ($p < 0.01$, Figure 6B).

Although the inhibitory effect of SIs on SW620 cells and the protection of NCM460 cells after 24 h treatment were not as good as expected, we continued culturing NCM460 cells after 24 h treatment for 9 days. As a result, we found that the abovementioned unfavorable effects of NCM460 persisted. The 12.5 and 50 μM concentrations could increase the GIN ($p < 0.05$, Figure 7A) of NCM460 cells, with the 12.5 μM concentration also inhibiting cell apoptosis ($p < 0.05$, Figure 7D), whereas there was no significant effect on NDI ($p > 0.05$, Figure 7C).

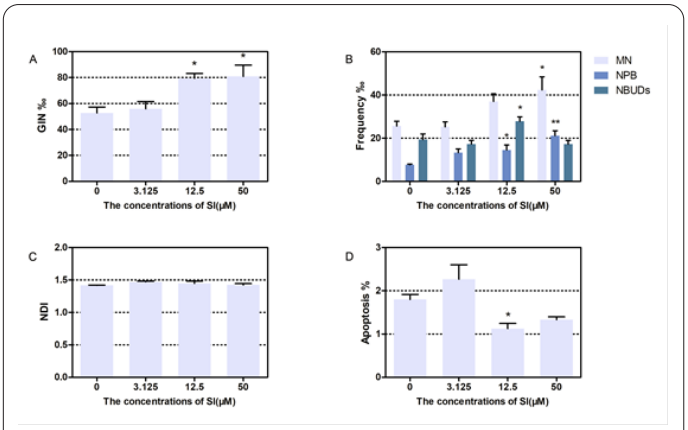

Figure 7. Influence of SI treatment for 9 days on the GIN, NDI, and apoptosis of NCM460 cells. NCM460 cells with a seeding density of 1 × 10^5/mL were incubated without or with SIs (3.125, 12.5, and 50 μM) for 24 h, and then continued culturing for 9 days. GIN (A), cell indicators (B), NDI (C), and apoptosis (D) were determined using the CBMN-Cyt assay; * $p < 0.05$, ** $p < 0.01$ vs. untreated control.

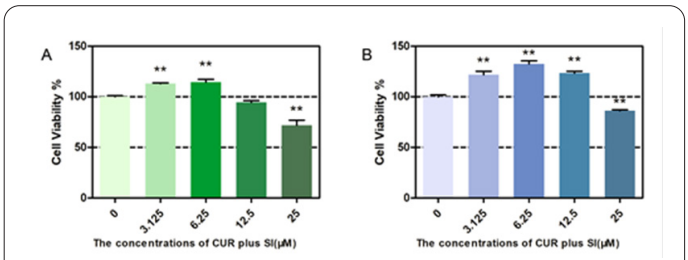

Figure 8. Influence of CUR plus SI treatment on the viability of SW620 (A) and NCM460 (B) cells for 24 h. The two cell lines with a seeding density of 2.5 × 10^4/mL were incubated without or with CUR plus SIs (3.125–25 μM) for 24 h, and then cell viability was determined using the MTT assay; * $p < 0.05$, ** $p < 0.01$ vs. untreated control.

CUR plus SIs can promote cell viability in SW620 and NCM460 cells

Furthermore, we were interested in the combined effect of CUR plus SIs to explore whether the anticancer and beneficial effects of CUR could be maximized through synergism with SI. Therefore, we administered a combination of CUR plus SIs in equal volume (1:1) at different concentrations (3.125–25 μM) to SW620 or NCM460 cells. With the exception of the 25 μM concentration, which had a certain inhibitory effect on the two cells ($p < 0.01$, Figure 8), the other concentrations (3.125 and 6.25 μM) could significantly promote the proliferation of SW620 and NCM460 cell lines ($p < 0.01$, Figure 8). This result was completely opposite to the effect of individual CUR or SI treatment.

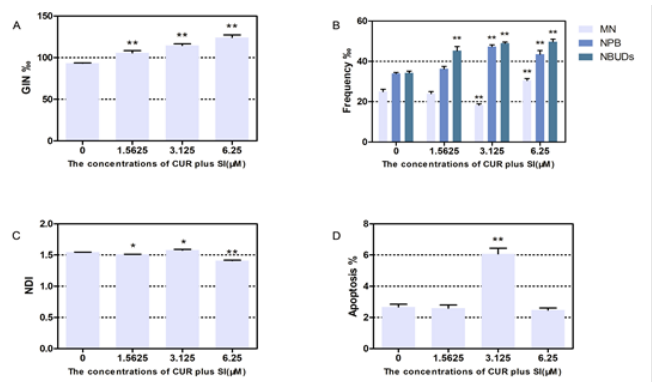

Figure 9. Influence of CUR plus SI treatment on the GIN, NDI, and apoptosis of SW620 cells for 24 h. SW620 cells with a seeding density of 1 × 10^5/mL were incubated without or with CUR plus SIs (1.5625, 3.125, and 6.25 μM) for 24 h. GIN (A), cell indicators (B), NDI (C), and apoptosis (D) were determined using the CBMN-Cyt assay; * $p < 0.05$, ** $p < 0.01$ vs. untreated control.

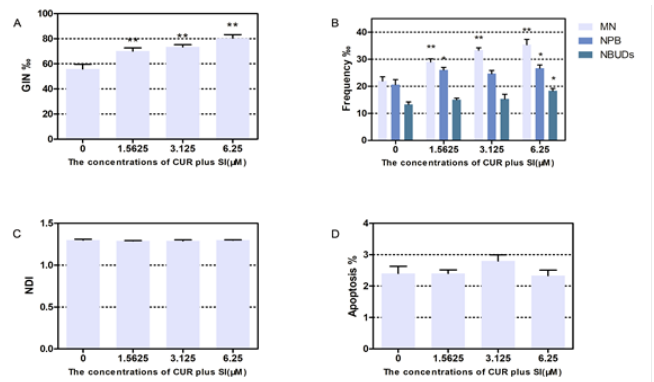

Figure 10. Influence of CUR plus SI treatment on the GIN, NDI, and apoptosis of NCM460 cells for 24 h. NCM460 cells with a seeding density of 1 × 10^5/mL were incubated without or with CUR plus SIs (1.5625, 3.125, and 6.25 μM) for 24 h. GIN (A), cell indicators (B), NDI (C), and apoptosis (D) were determined using the CBMN-Cyt assay; * $p < 0.05$, ** $p < 0.01$ vs. untreated control.

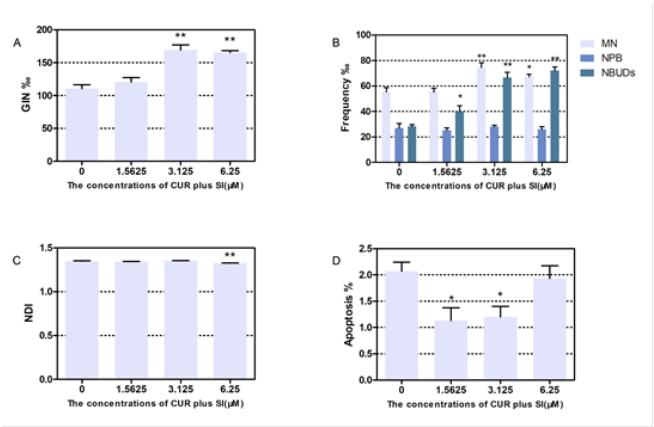

Figure 11. Influence of CUR plus SI treatment for 9 days on the GIN, NDI, and apoptosis of NCM460 cells. NCM460 cells with a seeding density of 1 × 10^5/mL were incubated without or with CUR plus SIs (1.5625, 3.125, and 6.25 μM) for 24 h, and then continued culturing for 9 days. GIN (A), cell indicators (B), NDI (C), and apoptosis (D) were determined using the CBMN-Cyt assay; * $p < 0.05$, ** $p < 0.01$ vs. untreated control.

Effect of CUR plus SIs on GIN in SW620 and NCM460 cells

We expected the combined effect of CUR plus SIs to be synergistic. As shown in Figures 1 and 8, the toxic effects of CUR and SIs on cells were slightly different in a dose-dependent manner. Therefore, for the CBMN-Cyt assay, in addition to choosing 3.125 μM and 6.25 μM concentrations for the MTT experiment (Figure 8), we explored a lower concentration (1.5625 μM) to obtain the expected results. The CBMN-Cyt assay was performed after the cells were treated with different concentrations of CUR plus SIs for 24 h. We found that 1.5625–6.25 μM could significantly increase cell GIN in SW620 ($p < 0.01$, Figure 9A), mainly represented by increases in NPB and NBUDs ($p < 0.01$, Figure 9B). Concentrations of 1.5625 and 6.25 μM could reduce NDI ($p < 0.05$, Figure 9C), whereas the 3.125 μM concentration could also induce apoptosis ($p < 0.05$, Figure 9D). In the same experiment, we also found that different concentrations of CUR plus SIs could increase the GIN of NCM460 cells ($p < 0.01$, Figure 10A). The increase in MN was most significant ($p < 0.01$, Figure 10B), whereas there were no significant effects on NDI or apoptosis ($p > 0.05$, Figure 10C, D).

Furthermore, we explored the combined maintenance effect of CUR plus SIs. After 24 h of CUR plus SI treatment, the NCM460 cells were cultured for 9 days. According to the results of the CBMN-Cyt assay, the 3.125 and 6.25 μM concentrations could significantly increase the GIN of NCM460 cells ($p < 0.01$, Figure 11A), mainly through significant changes in MN and NBUDs ($p < 0.05$, Figure 11B). Whereas the 6.25 μM concentration could reduce NDI ($p < 0.01$, Figure 11C), the 1.5625 and 3.125 μM concentrations could inhibit cell apoptosis ($p < 0.05$, Figure 11D).

Discussion

The ultimate goal of cell division for most noncancerous somatic cells is to accurately duplicate the genome and then evenly divide the duplicated genome into two daughter cells. This ensures that the daughter cells have exactly the same genetic material as their parent cell. Failure to achieve this purpose or an abnormally high frequency of errors during this process will result in various forms of genomic alterations in the daughter cells. These alterations include but are not limited to, various forms of mutations on specific genes, amplifications, deletions or rearrangements of chromosome segments, and gain or loss of an entire chromosome. The accumulation of these genomic alterations may cause dysregulation of cell division, an imbalance between cell growth and death, and cancer. GIN is defined as a process prone to genomic changes or an increased propensity for genomic alterations. Thus, GIN plays a key role in health maintenance, aging, and disease progression. A large rate of cell death destroys the homeostasis of the body's tissues, leading to a series of diseases, such as many monogenic diseases, autism, schizophrenia, epilepsy, emphysema, Parkinson's disease, Alzheimer's disease, and aging (30-33). This study analyzed the mechanism underlying the effects of CUR or SIs on the proliferation of SW620 cells from the perspective of cell GIN changes, revealing that CUR (12.5 μM) could increase the GIN of SW620 cells and promote cell apoptosis while promoting the proliferation and decreasing the GIN of NCM460 cells to a certain extent. Several concen-

trations (3.125, 6.25, 50, and 100 μM) of SIs had a certain degree of inhibition toward SW620 cell growth; for other concentrations selected in the experiment (3.125–50 μM), there was no expected effect on GIN and apoptosis in NCM460 cells, albeit a slight positive effect on NDI.

CUR is the rhizome extract of herb turmeric. It is widely used as a food pigment. It has a positive effect on arthritis, metabolic syndrome, obesity, and neurodegenerative diseases (34). Furthermore, it has also received attention from researchers due to its broad-spectrum antitumor activity. Some researchers have confirmed that it can prevent and treat prostate cancer by targeting the androgen receptor pathway (35). Both *in vivo* and *vitro* experiments have confirmed that it can improve the degree of genomic methylation in breast cancer cell lines (MDA-MB-231, MCF-7, T47D, etc.), thereby improving breast cancer preventive treatment and chemotherapy sensitivity (36). Therefore, this study aimed to detect whether the inhibitory effect of CUR on SW620 was through increasing GIN. The results confirmed our conjecture that CUR promotes apoptosis by increasing the GIN of SW620 cells, thus achieving the purpose of suppressing tumors. In addition to testing the antitumor activity of CUR, this study tested the effect on matched normal cells. It was found that CUR had a strong inhibitory activity on the proliferation of SW620 cells, along with a good effect on the colorectal epithelial cell line NCM460, exhibiting the ability to reduce GIN and promote cell proliferation. The experimental results are consistent with the related literature. Therefore, we speculate that CUR confers a certain degree of protection to intestinal epithelial tissue, in addition to its strong anti-colorectal tumor ability. However, the current experimental results are limited to in vitro settings, using individual cell types. It is necessary to further expand the investigation to additional types of tumor cells and conduct extensive in vivo experiments to explore their reliability and safety. At present, CUR-related drugs are undergoing clinical research (37), effectively showing that CUR has good application prospects.

SIs are the products of secondary metabolites naturally formed during the growth of plant soybeans, which are also called soybean phytoestrogens. They have the ability to reduce glucose absorption (38) and the risk of cardiovascular disease (21). Some scholars found that they also have biological effects such as combating atherosclerosis (21), improving female menopausal symptoms (18, 39) and preventing tumors (40). Through further research, it has been discovered that SIs play an important role in tumor prevention and treatment. Using an animal model, it was discovered that SIs could inhibit tumor cell proliferation and migration, thereby inhibiting tumor development (40) by activating ATM and P53 and other tumor suppressor gene-related pathways, inducing cell-cycle arrest and/or apoptosis, as well as inhibiting human colorectal cancer cell proliferation (41). However, large amounts of SIs may also result in negative effects, such as digestive tract reactions or allergic reactions, in addition to influencing sexual development in animals (42) and promoting the progression of prostate cancer in patients (43). Therefore, in-depth research on its anticancer activity and safety effects is particularly important. This study, from the perspective of cellular GIN, analyzed the molecular mechanism and safety effects of SIs against colorectal cancer. The experimental results revealed that, although SI treatment of SW620 cells could increase cell GIN, this was not reflected in the level of cell apoptosis. With regard to NCM460 cells, SIs treatment not only increased cell GIN, but also had a certain promotion effect on cell apoptosis. Both effects continued to manifest 9 days later. Although SIs have the potential to increase GIN in colorectal cancer, they can negatively impact normal cells. These results were also reported in other studies. The beneficial or anticancer effects differ to a certain extent according to drug concentration, sometimes even exhibiting opposite effects (44, 45). Therefore, with regard to the anticancer mechanism and safety effects of SI, investigations need to be expanded to other types of candidate tumor cells, while experimental conditions need to be refined and other strategies need to be explored.

The experimental design of the mixed treatment using CUR plus SI was based on the good anticancer activity of CUR and the ability of a low concentration of SIs to change GIN. Therefore, we aimed to test whether CUR plus SIs could produce a synergistic effect. Accordingly, we combined SIs and CUR in equal volumes (1.5625, 3.125, and 6.25 μM) using low concentrations of SIs, so as to achieve a better anti-colorectal cancer effect. However, the results revealed that combining the two treatments did not produce a synergistic effect on cells, instead causing the loss of their independent antitumor effects. Combinations are sometimes less effective than monotherapy (46), although better results are typically more common (47-50). More in-depth investigations are needed to clarify the specific relationship of CUR and SIs.

In summary, this study analyzed the effects of two plant active ingredients on the proliferation of normal and cancer cells from the perspective of GIN, providing a theoretical and scientific basis for their use in health maintenance and tumor adjuvant therapy. In our research, we found that the strong anticancer activity of CUR is consistent with the existing literature, while its safety effect in normal tissues is also an important discovery. However, these results were limited to colon cells cultured in vitro; thus, whether uniform results can be obtained in vivo or in other cell types remains to be further explored.

References

1. Panche AD, Diwan AD, Chandra SR. Flavonoids: an overview. J Nutr Sci. 2016(5):1-15.
2. Varghese E, Samuel SM, Abotaleb M, Cheema S, Mamtani R, Busselberg D. The "Yin and Yang" of Natural Compounds in Anticancer Therapy of Triple-Negative Breast Cancers. Cancers (Basel). 2018(10):346.
3. Costa A, Bonner MY, Arbiser JL. Use of Polyphenolic Compounds in Dermatologic Oncology. Am J Clin Dermatol. 2016;17(4):369-85.
4. Serino A, Salazar G. Protective Role of Polyphenols against Vascular Inflammation, Aging and Cardiovascular Disease. Nutrients. 2018;11(1).
5. Briguglio G, Costa C, Pollicino M, Giambò F, Catania S, C. F. Polyphenols in cancer prevention: New insights (Review). Int J Funct Nutr. 2020(1).
6. Giuliano C, Cerri S, Blandini F. Potential therapeutic effects of polyphenols in Parkinson's disease: in vivo and in vitro pre-clinical studies. Neural Regen Res. 2021;16(2):234-41.
7. Zhou Y, Zheng J, Li Y, Xu DP, Li S, Chen YM, et al. Natural Polyphenols for Prevention and Treatment of Cancer. Nutrients. 2016;8(8).

8. Arora I, Sharma M, Tollefsbol TO. Combinatorial Epigenetics Impact of Polyphenols and Phytochemicals in Cancer Prevention and Therapy. Int J Mol Sci. 2019;20(18).
9. Bian Y, Wei J, Zhao C, Li G. Natural Polyphenols Targeting Senescence: A Novel Prevention and Therapy Strategy for Cancer. Int J Mol Sci. 2020;21(2).
10. Meng B, Li J, Cao H. Antioxidant and antiinflammatory activities of curcumin on diabetes mellitus and its complications. Curr Pharm Des. 2013;19(11):2101-13.
11. Sahin Kavaklı H, Koca C, Alıcı O. Antioxidant effects of curcumin in spinal cord injury in rats. Ulus Travma Acil Cerrahi Derg. 2011;17(1):14-8.
12. Jin W, Wang J, Zhu T, Yuan B, Ni H, Jiang J, et al. Anti-inflammatory effects of curcumin in experimental spinal cord injury in rats. Inflamm Res. 2014;63(5):381-7.
13. De R, Kundu P, Swarnakar S, Ramamurthy T, Chowdhury A, Nair GB, et al. Antimicrobial activity of curcumin against Helicobacter pylori isolates from India and during infections in mice. Antimicrob Agents Chemother. 2009;53(4):1592-7.
14. Liu CH, Huang HY. Antimicrobial activity of curcumin-loaded myristic acid microemulsions against Staphylococcus epidermidis. Chem Pharm Bull. 2012;60(9):1118-24.
15. Shehzad A, Lee J, Lee YS. Curcumin in various cancers. Biofactors. 2013;39(1):56-68.
16. Trujillo J, Chirino YI, Molina-Jijón E, Andérica-Romero AC, Tapia E, Pedraza-Chaverrí J. Renoprotective effect of the antioxidant curcumin: Recent findings. Redox Biol. 2013;1(1):448-56.
17. Kiso Y, Suzuki Y, Watanabe N, Oshima Y, Hikino H. Antihepatotoxic principles of Curcuma longa rhizomes. Planta Med. 1983;49(3):185-7.
18. Tit DM, Bungau S, Iovan C, Nistor Cseppento DC, Endres L, Sava C, et al. Effects of the Hormone Replacement Therapy and of Soy Isoflavones on Bone Resorption in Postmenopause. J Clin Med. 2018;7(10).
19. Jayagopal V, Albertazzi P, Kilpatrick ES, Howarth EM, Jennings PE, Hepburn DA, et al. Beneficial effects of soy phytoestrogen intake in postmenopausal women with type 2 diabetes. Diabetes Care. 2002;25(10):1709-14.
20. Messina M. Soy foods, isoflavones, and the health of postmenopausal women. Am J Clin Nutr. 2014;100(1):4.
21. Ramdath DD, Padhi EM, Sarfaraz S, Renwick S, Duncan AM. Beyond the Cholesterol-Lowering Effect of Soy Protein: A Review of the Effects of Dietary Soy and Its Constituents on Risk Factors for Cardiovascular Disease. Nutrients. 2017;9(4).
22. Wada K, Nakamura K, Tamai Y, Tsuji M, Kawachi T, Hori A, et al. Soy isoflavone intake and breast cancer risk in Japan: from the Takayama study. Int J Cancer. 2013;133(4):952-60.
23. Abdelrazek HMA, Mahmoud MMA, Tag HM, Greish SM, Eltamany DA, Soliman MTA. Soy Isoflavones Ameliorate Metabolic and Immunological Alterations of Ovariectomy in Female Wistar Rats: Antioxidant and Estrogen Sparing Potential. Oxid Med Cell Longev. 2019;10(5713606).
24. Anderson JJ, Anthony MS, Cline JM, Washburn SA, Garner SC. Health potential of soy isoflavones for menopausal women. Public Health Nutr. 1999;2(4):489-504.
25. Guo X, Ni J, Dai X, Zhou T, Yang G, Xue J, et al. Biphasic regulation of spindle assembly checkpoint by low and high concentrations of resveratrol leads to the opposite effect on chromosomal instability. Mutat Res Genet Toxicol Environ Mutagen. 2018;825:19-30.
26. Ni J, Guo X, Wang H, Zhou T, Wang X. Differences in the Effects of EGCG on Chromosomal Stability and Cell Growth between Normal and Colon Cancer Cells. Molecules. 2018;23(4).
27. Guo X, Wang H, Ni J, Liang Z, Wu X, Xue J, et al. Geraniin selectively promotes cytostasis and apoptosis in human colorectal cancer cells by inducing catastrophic chromosomal instability. Mutagenesis. 2018;33(4):271-81.
28. Guo X, Ni J, Liu X, Xue J, Wang X. Phyllanthus emblica L. fruit extract induces chromosomal instability and suppresses necrosis in human colon cancer cells. Int J Vitam Nutr Res. 2013;83(5):271-80.
29. Fenech M. Cytokinesis-block micronucleus assay evolves into a "cytome" assay of chromosomal instability, mitotic dysfunction and cell death. Mutat Res. 2006;600(1-2):58-66.
30. Ferguson LR, Chen H, Collins AR, Connell M, Damia G, Dasgupta S, et al. Genomic instability in human cancer: Molecular insights and opportunities for therapeutic attack and prevention through diet and nutrition. Semin Cancer Biol. 2015;35(10):11.
31. Stankiewicz P, Lupski JR. Structural variation in the human genome and its role in disease. Annu Rev Med. 2010;61:437-55.
32. Misteli T. Higher-order genome organization in human disease. Cold Spring Harb Perspect Biol. 2010;2(8):30.
33. López-Otín C, Blasco MA, Partridge L, Serrano M, Kroemer G. The hallmarks of aging. Cell. 2013;153(6):1194-217.
34. Giordano A, Tommonaro G. Curcumin and Cancer. Nutrients. 2019;11(10).
35. Javed Z, Khan K, Rasheed A, Sadia H, Shahwani MN, Irshad A, et al. Targeting androgen receptor signaling with MicroRNAs and Curcumin: a promising therapeutic approach for Prostate Cancer Prevention and intervention. Cancer Cell Int. 2021;21(1):021-01777.
36. Fabianowska-Majewska K, Kaufman-Szymczyk A, Szymanska-Kolba A, Jakubik J, Majewski G, Lubecka K. Curcumin from Turmeric Rhizome: A Potential Modulator of DNA Methylation Machinery in Breast Cancer Inhibition. Nutrients. 2021;13(2).
37. Sminia P, van den Berg J, van Kootwijk A, Hageman E, Slotman BJ, Verbakel W. Experimental and clinical studies on radiation and curcumin in human glioma. J Cancer Res Clin Oncol. 2021;147(2):403-9.
38. Ademiluyi AO, Oboh G. Soybean phenolic-rich extracts inhibit key-enzymes linked to type 2 diabetes (α-amylase and α-glucosidase) and hypertension (angiotensin I converting enzyme) in vitro. Exp Toxicol Pathol. 2013;65(3):305-9.
39. Daily JW, Ko BS, Ryuk J, Liu M, Zhang W, Park S. Equol Decreases Hot Flashes in Postmenopausal Women: A Systematic Review and Meta-Analysis of Randomized Clinical Trials. J Med Food. 2019;22(2):127-39.
40. Gómez-Zorita S, González-Arceo M, Fernández-Quintela A, Eseberri I, Trepiana J, Portillo MP. Scientific Evidence Supporting the Beneficial Effects of Isoflavones on Human Health. Nutrients. 2020;12(12).
41. Zhang Z, Wang CZ, Du GJ, Qi LW, Calway T, He TC, et al. Genistein induces G2/M cell cycle arrest and apoptosis via ATM/p53-dependent pathway in human colon cancer cells. Int J Oncol. 2013;43(1):289-96.
42. Jargin SV. Soy and phytoestrogens: possible side effects. Ger Med Sci. 2014;15(12).
43. Zhang HY, Cui J, Zhang Y, Wang ZL, Chong T, Wang ZM. Isoflavones and Prostate Cancer: A Review of Some Critical Issues. Chin Med J. 2016;129(3):341-7.
44. Bhattacharya U, Mukhopadhyay S, Giri AK. Comparative antimutagenic and anticancer activity of three fractions of black tea polyphenols thearubigins. Nutr Cancer. 2011;63(7):1122-32.
45. Ilieș M, Uifălean A, Pașca S, Dhople VM, Lalk M, Iuga CA, et al. From Proteomics to Personalized Medicine: The Importance of Isoflavone Dose and Estrogen Receptor Status in Breast Cancer Cells. J Pers Med. 2020;10(4).
46. Mahbub AA, Le Maitre CL, Haywood-Small SL, Cross NA,

Jordan-Mahy N. Polyphenols act synergistically with doxorubicin and etoposide in leukaemia cell lines. Cell Death Discov. 2015;1(15043).

47. Yousefi, B., Safari, H. Evaluation of Essential Oil, Morphological Traits and Crop Yield in Satureja mutica Fisch. & C. A. Mey. in Rainfed Cultivation under Different Row Spacing. Agrotech Ind Crops 2022; 2(4): 187-197. doi: 10.22126/atic.2023.8424.1074.
48. Chaghakaboodi, Z., Nasiri, J., Farahani, S. Fumigation Toxicity of the Essential Oils of Ferula persica against Tribolium castaneum and Ephestia kuehniella. Agrotech Ind Crops 2022; 2(3): 123-130. doi: 10.22126/atic.2022.8344.1068
49. Calniquer G, Khanin M, Ovadia H, Linnewiel-Hermoni K, Stepensky D, Trachtenberg A, et al. Combined Effects of Carotenoids and Polyphenols in Balancing the Response of Skin Cells to UV Irradiation. Molecules. 2021;26(7).
50. Amin AR, Wang D, Zhang H, Peng S, Shin HJ, Brandes JC, et al. Enhanced anti-tumor activity by the combination of the natural compounds (-)-epigallocatechin-3-gallate and luteolin: potential role of p53. J Biol Chem. 2010;285(45):34557-65.