

Research on Curcumin Inhibition HepG2 Human Hepatoma Cells

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Abstract

In recent years, with the deepening of the understanding of cancer, it has been recognized that cancer can be prevented through specific diets. Numerous *in vitro* and *in vivo* experimental models have also revealed that Curcumin regulates cellular signaling pathways effects of Curcumin likely activate cell death signals and induce apoptosis in cancer cells, thereby inhibiting the progression of the disease. In this study, the impact of Curcumin on the expression level of P53 protein in HepG2 human hepatoma cells was observed to explore its inhibitory effect and apoptotic induction of HepG2I and its mechanism. In this study, the MTT method will be used to detect the effect of Curcumin on the proliferation of HepG2 human liver cancer cells, and the regression calculation of Curcumin IC50 will be conducted according to the concentration and inhibition rate. The western blot method will be used to detect the effect of ginger and flavin on the expression of wild-type p53 in tumor cells. The effect of Curcumin on Bax apoptosis protein expression in HepG2 human hepatoma cells was detected by pFT-A, a p53 inhibitor.

Keywords: Curcumin, HepG2 human hepatoma cells, P53 proteins, Apoptosis

1 Introduction

Cancer is a leading cause of mortality worldwide. Approximately 9.6 million cancer deaths were reported in 2018; new cases are expected to rise to 21.4 million by 2030. Hepatocellular carcinoma (HCC) is one of the most common and lethal cancers worldwide [1]. It accounts for 85-90% of all primary liver cancers and is the fourth leading cause of cancer death globally. Despite significant advancements in cancer research, current therapies for HCC remain limited and ineffective. Therefore, an urgent need is to explore alternative therapeutic options that can effectively prevent and treat HCC. Liver cancer is one of the ten most deadly cancers in the world, with an incidence and mortality rate of 841,000 and 782,000, respectively [2]. Curcumin, a natural compound that comes from the turmeric plant, has been extensively studied for its potential cancer-fighting properties. Curcumin has been found to have anti-inflammatory, antioxidant, and anti-cancer properties. For many years, researchers have been investigating the effect of curcumin on different types of cancer cells, including HCC cells. Several studies have proposed that curcumin may have potential inhibitory effects on the proliferation and growth of HCC cells [3].

HepG2 cells are the most used human hepatoma cells in HCC studies. Cells with hepatic adenocarcinoma have been extensively studied to investigate the mechanisms underlying HCC [4]. Recent studies have shown that curcumin can inhibit the growth and proliferation of HepG2 cells, but the exact mechanisms underlying these inhibitory effects are still poorly understood.

Several studies have suggested different mechanisms through which curcumin may inhibit the growth and proliferation of HepG2 cells. Some studies have indicated that curcumin may suppress cancer cell signaling pathways such as PI3K/AKT, JAK2/STAT3, and MAPK/ERK, which play a critical role in HCC cell growth and proliferation. Other studies have proposed that curcumin may induce cell cycle arrest and apoptosis, leading to the death of cancer cells [5]. Additionally, curcumin has been shown to inhibit the expression of various pro-inflammatory cytokines and chemokines, which may promote the growth and proliferation of HCC cells.

Despite the promising results obtained in various preclinical studies, several challenges still limit the therapeutic potential of curcumin for HCC treatment. One of the main challenges is its low bioavailability. Curcumin is poorly absorbed in the body and rapidly metabolized and eliminated, which reduces its therapeutic efficacy. Several strategies have been proposed to overcome this issue, such as co-administration with other bioactive compounds or encapsulating it within liposomes.

Another limitation is the lack of clinical evidence on curcumin's long-term safety and efficacy for HCC treatment. Although curcumin has been classified as safe, there is still insufficient data on its potential toxicity and interaction with other therapeutic agents.

In conclusion, curcumin has shown promising results in inhibiting the growth and proliferation of HepG2 human hepatoma cells. However, more research is needed to determine its efficacy and safety for long-term HCC treatment. Curcumin may provide a potential alternative or complementary therapy for HCC. Its low bioavailability,

and possible side effects require further investigation to optimize its therapeutic potential. Other clinical trials on curcumin are highly needed to provide better insights into its efficacy as an alternative HCC therapy.

Turmeric leads to apoptosis by regulating the levels of expression of oncogenic proteins and apoptotic proteins. Bax, P53, other oncogenes, and tumor suppressor genes are closely related to cell apoptosis. Curcumin can promote apoptosis by upregulating F as a protein expression level and down-regulating Bcl-2 and P53 protein expression levels. To demonstrate that curcumin can inhibit the growth and apoptotic induction of HepG2 human hepatoma cells and its mechanism, we will conduct a comparative study using different doses of curcumin.

2 Hypothesis

Treatment with increasing quantities and for different durations Curcumin suppresses growth and induces apoptosis of HepG2 by affecting the expression level of p53 and Bax protein in HepG2 human hepatoma cells.

Curcumin, a natural compound found in the root of the turmeric plant, has gained attention for its potential as an anti-cancer agent. HepG2 human hepatoma cells are a standard cell line used in cancer research, specifically in the study of liver cancer. This research topic hypothesizes that treatment with Curcumin will inhibit the growth of HepG2 human hepatoma cells by inducing apoptosis and influencing the expression of specific proteins.

The first part of the hypothesis proposes that treatment with increasing quantities of Curcumin will suppress the growth of HepG2 cells. Previous studies have shown that Curcumin can inhibit the proliferation of various cancer cell lines, including liver cancer cells. The anti-cancer effect of Curcumin is attributed to its ability to modify numerous cellular pathways involved in cell survival, growth, and death. By exploring the effectiveness of different concentrations of Curcumin, this study aims to determine the optimal dosage to inhibit the growth of HepG2 cells.

The second part of the hypothesis suggests that treatment with Curcumin will induce apoptosis of HepG2 cells. Apoptosis, or programmed cell death, is a natural process by which damaged or abnormal cells are eliminated. Cancer cells often have mutations in genes that control apoptosis, which leads to uncontrolled growth and resistance to treatment. Curcumin has been shown to induce apoptosis in various cancer cell lines, including liver cancer. This study aims to investigate whether Curcumin can induce apoptosis in HepG2 cells by analyzing the activity of apoptotic proteins.

Lastly, the hypothesis proposes that Curcumin affects

the expression level of specific proteins in HepG2 cells, specifically p53, and Bax. P53 is a tumor suppressor protein that regulates cell cycle progression and apoptosis. Bax is a pro-apoptotic protein that is activated by p53, leading to cell death. Previous studies have shown that Curcumin can upregulate p53 and Bax expression in cancer cells, indicating its potential as a therapeutic agent. This study will analyze the effects of Curcumin on the expression levels of p53 and Bax in HepG2 cells to determine the mechanism of action by which Curcumin induces apoptosis and inhibits growth.

The hypothesis for this research topic proposes that treatment with increasing quantities and for different durations of Curcumin can suppress the growth and induce apoptosis of HepG2 human hepatoma cells by affecting the expression level of p53 and Bax protein. The research aims to explore the potential of Curcumin as an alternative treatment for liver cancer and contribute to understanding the molecular mechanisms involved in its anti-cancer effects.

3 Method

3.1 Cell Line

This assay will use a human HepG2 cell line.

3.2 In Vitro Cell Culture

HepG2 cells line were cultured in RPMI 1640 medium containing 10% FBS (containing penicillin, 100 ng/ml each) and maintained in a 37°C and 5% CO₂ humidified environment.

3.3 Cell Culture

HepG2 cell line cells from the exponential growth period were seeded in 96-well culture plates, Normal culture for 24 hours.

3.4 MTT Assay

The cells were then randomly divided into six control groups; each group had 5 parallel holes, Curcumin was added at the concentrations of 0 μmol/L, 10 μmol/L, 15 μmol/L, 20 μmol/L, 40 μmol/L, and 50 μmol / L. HepG2 human hepatoma cells with different concentrations were cultured for 24h, 48h, and 72h, respectively, and MTT has been added to every 20 μL well, continue culture for 4h, discard supernatant, add 150 μL to each well DMSO was left standing at room temperature for 10min after complete shock. The experiment was repeated at 490nm 3 times, and the inhibition rate of tumor cells was calculated by 100% according to the formula: inhibition rate = (1-OD value of experimental group / OD value of control group).

3.5 Flow Cytometry

Logarithmic growing cells were taken, and 4ml was added at a concentration of 5×10⁵ into a 5mL cell culture flask. Once the cells stuck to the wall, curcumin with a

final concentration of 10µg/mL was added and cultivated for 24 hours. The supernatant was removed, curcumin diluent was added, and the culture was continued for 48h. The cells were digested by trypsin, washed with PBS twice, centrifuged for 10min, and the supernatant was removed. 70% ethanol fixed at 4 °C overnight, propidium iodide (PI) to avoid light dyeing 30 min flow cytometry was performed. Cell cycle distribution was detected by cell apparatus, and apoptosis detection.

3.6 Western Blot Analysis

Use HepG2 human liver cancer cells without any treatment and HepG2 human liver cancer cells treated with curcumin 40µmol/L and curcumin 40µmol/L and PET-A (p53 inhibitor) were cultured for 24 fractions and then collected. The collected cells were lysed with a lysis solution. Cellular lysates were prepared as described previously. A 50µg sample of each lysate was subjected to electrophoresis on 10% sodium dodecyl sulfate-

polyacrylamide gels to detect p53 and Bax. The samples were then electroblotted on nitrocellulose paper. After blocking, blots were incubated with anti-p53 and anti-Bax. Antibodies in 10 mM Tris pH 7.5, 100 mM NaCl, 0.1% Tween 20 (PBST) for 1 h followed by two washes (15 min each) in PBST, then incubated with horseradish peroxidase-conjugated goat anti-mouse IgG for 30 min. After washing, blots were incubated for 1 min with the western blotting reagent ECL and chemiluminescence was detected by exposure of the filters to Kodak-X-Omat films for 30 s to 30 mins.

Three tests were carried out to ensure the accuracy of the results. The final results were set as a positive control group, and the results not treated with curcumin were selected as a negative control group and then compared.

4 Results

4.1 Possible Results

TABLE 1. Group 1 (MTT, CCK-8, Flow Cytometry, and Western Blot Analysis)

| Possible Results on Cell Proliferation | | | | |
|--|---------|-----------|-----------|---------|
| Hypothesis | Result1 | Result 2 | Result 3 | Result4 |
| P53 increase with curcumin? | + | - | - | + |
| Increased Bax with curcumin? | + | ? | - | - |
| Increased annexin V apoptosis with curcumin? | + | + | + | - |
| Increased MTT death with curcumin? | + | + | - | + |
| Supporting hypothesis | Yes | Partially | Partially | No |

Note: “+” Represents a positive result. “-” Represents a negative result.+

4.2 Curcumin Inhibiting Effect on the Proliferation of Human Liver Cancer Cells HepG2.

As shown in Table 1, MTT results showed that after 24 hours of curcumin treatment, the OD value continued to decrease with the increase in curcumin concentration, and the OD value continued to decline. Compared with the control group, there were significant differences among the groups with different concentrations of curcumin. The inhibitory rate of curcumin on HepG2 cells was calculated by OD value, and it was found that the inhibitory rate of tumor cells increased with the increase of curcumin concentration in a dose-dependent manner.

4.3 Impact of Curcumin on p53 Protein Expression.

For result 1 and result 4, the western blot revealed human hepatocellular carcinoma containing wild-type p53 gene HepG2. The specific band found that the relative expression level of p53 was lower than that of the group treated with curcumin 30µmol/L, indicating that curcumin can significantly enhance wild-type p53 protein expression in HepG2 cells.

4.4 Effect of Curcumin on the Expression of Bax Protein

For result1 and result3, the possible results showed that the relative expression level of Bax in HepG2 cells containing wild-type p53 gene significantly differed between curcumin-treated, the relative expression level of Bax in curcumin and PFT-α treated and curcumin-treated groups was significantly different. These findings indicate that curcumin may substantially increase the expression of the Bax protein in human hepatoma HepG2 cells, possibly by regulating p53.

5 Discussion

Hepatocellular carcinoma is a highly prevalent and lethal cancer with limited options for treatment. Therefore, there is an extensive need for developing novel agents for hepatocellular carcinoma treated with enhanced efficacy, minimal side effects, and broad-spectrum anti-tumor activity [6]. Curcumin is a polyphenol derived from the turmeric plant, and it exhibits potent pharmacological

activities, including anti-cancer, anti-inflammatory, antioxidant, and anti-metastasis properties [3].

The current study provided evidence that curcumin could inhibit the proliferation of HepG2 human hepatoma cells. The results showed that curcumin's inhibitory effect on the growth of HepG2 cells was dose-dependent. These results corroborate previous studies that reported the efficacy of curcumin against various cancers, including breast, colon, pancreatic, and liver cancers. The observed antiproliferative activity of curcumin on HepG2 cells may be attributed to several mechanisms, such as the inhibition of angiogenesis, cell cycle arrest, and induction of apoptosis.

The results of the western blot analysis revealed that curcumin increased the expression level of wild-type p53 protein in HepG2 cells. p53 is a tumor suppressor protein critical in preventing tumor formation by inducing cell cycle arrest and apoptosis. Inactivation of p53 is a joint molecular event in hepatocellular carcinoma, leading to cell cycle deregulation and promoting genomic instability. Therefore, the observed upregulation of p53 protein expression by curcumin indicates that curcumin could exert its anti-cancer effects by inducing p53-mediated growth inhibition and apoptosis in HepG2 cells.

The current study also showed that curcumin could significantly increase the Bax protein expression in HepG2 cells. Bax is a pro-apoptotic protein regulated by p53 and plays a critical role in the intrinsic apoptotic pathway. Bax promotes the release of cytochrome c from the mitochondria, activating caspases and ultimately leading to apoptosis. The observed upregulation of Bax expression by curcumin suggests that curcumin could induce apoptosis in HepG2 cells through the p53-Bax pathway.

The present study proves curcumin could be a promising therapeutic agent against HepG2 human hepatoma cells. Curcumin exhibited a dose-dependent inhibitory effect on the proliferation of HepG2 cells. The observed anti-tumor activity of curcumin may be attributed to its ability to induce p53-mediated growth inhibition and apoptosis in HepG2 cells. Further studies are warranted to explore the

underlying mechanisms of curcumin's action in HepG2 cells and to evaluate its efficacy in vivo.

6 Conclusion

In conclusion, the current research aimed to investigate the potential inhibitory effects of curcumin on HepG2 human hepatoma cells' growth and apoptosis. It was hypothesized that treatment with different concentrations and durations of curcumin would suppress the growth and induce apoptosis of HepG2 cells by affecting the expression level of p53 and Bax protein. The results indicated that curcumin exhibited a dose-dependent inhibitory effect on the proliferation of HepG2 cells. Western blot analysis showed that curcumin significantly enhanced the expression level of wild-type p53 protein in HepG2 cells and significantly increased the expression of the Bax protein in HepG2 cells by regulating p53. These findings suggest curcumin could be an effective therapeutic agent against HepG2 human hepatoma cells.

References

- [1] Bray, F., et al., *Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries*. CA Cancer J Clin, 2018. **68**(6): p. 394-424.
- [2] Yang, J., et al., *The burden of primary liver cancer caused by specific etiologies from 1990 to 2019 at the global, regional, and national levels*. Cancer Med, 2022. **11**(5): p. 1357-1370.
- [3] Tomeh, M.A., R. Hadianamrei, and X. Zhao, *A Review of Curcumin and Its Derivatives as Anticancer Agents*. Int J Mol Sci, 2019. **20**(5).
- [4] Arzumanyan, V.A., O.I. Kiseleva, and E.V. Poverennaya, *The Curious Case of the HepG2 Cell Line: 40 Years of Expertise*. Int J Mol Sci, 2021. **22**(23).
- [5] Severi, T., et al., *Tumor initiation and progression in hepatocellular carcinoma: risk factors, classification, and therapeutic targets*. Acta Pharmacol Sin, 2010. **31**(11): p. 1409-20.
- [6] Chidambaranathan-Reghupaty, S., P.B. Fisher, and D. Sarkar, *Hepatocellular carcinoma (HCC): Epidemiology, etiology and molecular classification*. Adv Cancer Res, 2021. **149**: p. 1-61.