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ORIGINAL ARTICLE: CLINICAL TRANSLATIONAL THERAPEUTICS

Upregulation of p53 Expression in Patients with Colorectal Cancer by Administration of Curcumin

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Biological therapies can be beneficial in cancer patients. The present study aims to examine the inhibitory mechanism of curcumin on cancer cells in patients with colorectal cancer. The results showed that curcumin administration increased body weight, decreased serum TNF-alpha levels, increased apoptotic tumor cells, enhanced expression of p53 molecule in tumor tissue, and modulated tumor cell apoptotic pathway. We conclude that the curcumin treatment improves the general health of patients with colorectal cancer via the mechanism of increased p53 molecule expression in tumor cells and consequently speeds up tumor cell apoptosis.

Keywords: Cancer; Colon; Apoptosis; Curcumin

INTRODUCTION

The radical removal of cancer relies on early diagnosis and surgical approaches, whereas the treatment for advanced cancer is still unsatisfactory (1, 2). Therefore, various remedies for the treatment of advanced cancer, such as radiotherapy (3), chemotherapy (2), and various biological therapies (4), have been tried in cancer clinic.

Colorectal cancer (CRC) is one of the leading causes of death; it is ranked as the fifth leading cause of death in China. The prevalence of CRC is actually rising despite advanced research being made in recent years. The pathogenesis of CRC is poorly understood. Presently, the 5-year survival rate with CRC is not satisfactory.

Cancer cachexia is common in cancer patients. It is a complex metabolic condition characterized by loss of skeletal muscles. In clinic, cancer patients with cachexia manifest as anemia, muscle wasting, reduced appetite, and altered immune function, which result in fatigue, diminished quality of life, and reduced survival. So far no specific remedies have been identified for cancer cachexia.

Curcumin is the principal curcuminoid of the popular Indian spice turmeric, which is a member of the ginger family (Zingiberaceae) (5). Over the last several centuries, studies have revealed important functions of curcumin. Extensive research has shown various actions of curcumin, such as anti-inflammatory, cytokines release, antioxidant, immunomodulatory, enhancing of the apoptotic process, and anti-angiogenic properties. Specifically, laboratory studies indicate that curcumin can upregulate the expression of p53 in cancer cells, and modulate some apoptosis-related molecules, such as Bax and Bcl-2, in cancer cells to induce cancer cell apoptosis (6–8).

There are not many publications on the effect of curcumin on suppression of cancer in clinic. Owing to side effects of chemotherapy and radiotherapy, some cancer patients would not accept these remedies during the period ahead of surgery. Therefore, for the present study we randomly selected a group of patients with CRC who received curcumin therapy after diagnosis and before surgery. The results showed that curcumin administration had measurable effect on weight loss, suppressed serum levels of tumor necrosis factor (TNF)-α, and induced cancer cell apoptosis.

MATERIALS AND METHODS

Reagents: Curcumin and Polymerase Chain Reaction (PCR) reagents were purchased from Sigma Aldrich (Shanghai, China). Curcumin vehicle was purchased from LG Biotech Ltd (Shanghai, China). Western blotting reagents were purchased from Invitrogen (Shanghai, China). Antibodies against TNF-α, p53, Bax, Bcl-2, and β-actin were purchased from Santa Cruz (Santa Cruz, CA, USA). Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) reagent kit and TNF-α ELISA kit were purchased from R&D System (Shanghai, China).

Patients: A total of 126 patients were diagnosed with CRC and were randomly selected and admitted to the 2nd Affiliated Hospital of Nanjing Medical University, Nanjing, Jiangsu, China between January 2000 and June 2007. The demographic data are presented in Table 1. All patients received primary surgical therapy. After surgery, patients received additional therapies, including radiotherapy (31 patients), chemotherapy (84 patients), and both radiotherapy

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Table 1. Demographic Data

Group	Curcumin	Vehicle
Sex		
Female	37	34
Male	26	28
pTNM stage	34–65 (62.3)	39–82 (64.1)
Stage I	9	13
Stage II	32	30
Stage III	16	14
Stage IV	6	6
Tumor location		
Right	28	26
Left	19	23
Rectum	16	14
Tumor size		
≤40 mm	28	31
>40 mm	35	32
Differentiation		
Good	21	17
Moderate	18	28
Poor	24	18

and chemotherapy (9 patients); 20 patients received no additional chemotherapy. The usage of human tissue for the study was approved by the Ethic Committee at Nanjing Medical University. Informed consent was signed by each patient. Patients receiving other therapeutic remedies were excluded from this study.

Administration with curcumin: The selected 126 CRC patients were randomly divided into two groups: the curcumin group and the vehicle group. The random numbers were generated by a computer; if a patient was assigned an even number, he was allocated to curcumin group; if it was an odd number, the patient was a control case in the vehicle group. The curcumin group patients received 360 mg (in capsule form) curcumin, thrice/day during the period ahead of surgery. As the waiting time varied from patient to patient, the treating time also varied between 10 days and 30 days (10–15 days: 12 patients, 16–20 days: 15 patients, 21–25 days: 18 patients, and 26–30 days: 18 patients). The vehicle group patients received vehicle (in capsule form) thrice/day during the period ahead of surgery.

Collection of colorectal cancer specimen: Two specimens were obtained from each patient: one was at the time of diagnosis (biopsy via colonoscopy), and another at the time of surgery (surgical removed of CRC tissue).

Enzyme-linked immunosorbent assay (ELISA): Blood samples were collected from each patient before and after the treatment. Sera were separated from blood, and serum levels of TNF-α were determined by ELISA following the manufacturer's instructions.

TUNEL assay: Apoptotic cells in CRC tissues were detected by TUNEL following the manufacturer's instructions.

Immunohistochemistry: p53 expression in tumor tissue was detected by immune staining following published procedures with modification (9). Briefly, cryosections were fixed by cold acetone for 20 min and blocked by 1% bovine serum albumin (BSA) for 30 min. Sections were incubated with anti-p53 antibody (1 μg/ml; or isotype IgG) at 4°C overnight. The sections were then incubated by the secondary antibody (1:300) for 1 hr at room temperature. Washing with phosphate buffered saline (PBS) was done in-between. Sections were observed under a microscope at a magnification of 200×.

Western blotting: Bax and Bcl-2 expressions in tumor tissue were detected by Western blotting following previous report with modification (10). Briefly, cellular extracts were separated by pre-cast gel and transferred onto nitrocellulose membrane. The membrane was blocked by 5% skimmed milk for 1 hr and incubated with the primary antibodies (1 μg/ml; or isotype IgG) at 4°C overnight. After washing, the membrane was incubated with horseradish peroxidase-labeled second antibody for 1 hr at room temperature. The blots were developed by Enhanced Chemiluminescence (ECL) reagent. The signal was recorded on an X-ray film.

Determination of DNA fragmentation in CRC tissue: Apoptosis in CRC tissue was determined by the DNA fragmentation assay. Briefly, total DNA was extracted from surgically removed cancer tissue. The extracted DNA (40 μg/lane) was subjected to electrophoresis on 1.5% agarose gels. The gels were stained with ethidium bromide and photographed.

Statistical analysis: Data were presented as mean ± SD. Difference between two groups was analyzed by Student's *t*-test (a paired *t*-test), or ANOVA if there are more than two groups. $p < .05$ was considered as significant.

RESULTS

Body weight increases in patients with colorectal cancer after curcumin treatment

Improvement in general health conditions of patients can facilitate anti-cancer treatment. Body weight is one of the important parameters reflecting the general health condition. Laboratory studies indicate that curcumin administration has anti-cancer effect (6–8). We postulate that curcumin ingestion may improve the general health of a cancer-laden body. In order to test the hypothesis, we selected 126 patients with CRC. During the period ahead of surgery, 63 patients were administrated curcumin orally at a dose of 360 mg/day; another 63 patients took vehicle in a double-blind way. The treatment lasted for 10–30 days. The treatment schedule is presented in Table 2. The body weight was recorded for each patient before and after the curcumin treatment. The results showed that curcumin administration significantly improved the body weight of patients with CRC as compared with the control group (Figure 1).

As the body weight change might be affected by other factors, such as calorie intake, diarrhea, and CRC-caused obstruction, we also considered these factors in the present study. As the calorie intake was reviewed and recorded by dieticians, no significant changes were noted in patients in either the curcumin group or the vehicle group. We did notice patients with diarrhea in both groups during the study period; 8 and 10 patients, respectively, had diarrhea in vehicle and curcumin groups ($p > .05$). No

Table 2. Time for Which Patients Received Treatment with Curcumin or Vehicle

Days	7	8	9	10	11	12	13	14	15	16	17	18
Curcumin	*3*	*11*	*2*	*5*	*1*	*1*		*3*	*4*	*4*		*3*
Vehicle	*4*	*14*	*2*	*1*	*4*		*4*		*3*	*2*	*3*	
Days	19	20	21	22	23	24	25	26	27	28	29	30
Curcumin	*1*	*4*	*1*	*4*	*4*		*3*	*1*	*3*	*3*	*1*	*1*
Vehicle	*5*		*4*		*4*		*3*	*5*	*1*	*1*	*2*	*1*

Curcumin: patients received curcumin treatment. Vehicle: Patients received vehicle treatment. The number in each grid represents the number of patient.

obstruction case was noticed in either group during the study period.

Serum level of TNF-α is suppressed in curcumin group patients

TNF-α is one of the major toxic factors secreted by cancer cells that have severe impact on human health, and was, at least partially, responsible for weight loss. The fact that curcumin administration improved the body weight of patients with CRC implies that the amount of TNF-α in a tumor-laden body may be reduced. Thus, we assessed the serum levels of TNF-α in CRC patients before and after the curcumin treatment. As shown by ELISA data, significant reduction of the serum TNF-α level was observed in the curcumin-treated patients, but not in those treated with vehicle (Figure 2). A correlation assay between body weight change and serum TNF-α level was performed. The results showed a negative correlation in patients with CRC ($r = 0.4064$, $p < .05$). The results indicate that the weight gain in CRC patients may be related to the suppression of serum TNF-α level.

Treatment with curcumin speeds up cancer cell apoptosis

One of the strategies of cancer therapy is to induce cancer cell apoptosis. We postulate that curcumin may speed up cancer cell apoptosis. In order to test the hypothesis, we examined apoptotic cells by the TUNEL staining and DNA fragmentation in CRC tissues before (the diagnostic biopsies) and after (surgical removal of CRC tissue) the curcumin treatment. As shown by the TUNEL staining, few apoptotic cells were detected in CRC tissues before the curcumin treatment, whereas abundant apoptotic cells were observed after the treatment; the apoptotic cell frequency did not change much in the vehicle group (Figure 3(a) and (b)). The results were supported by the DNA fragmentation assay that showed much more DNA fragmentation in CRC samples from patients treated with curcumin than samples from the same group before curcumin treatment and those treated with vehicle (Figure 3(c)).

Administration of curcumin increases the expression of p53 in colorectal cancer

P53 is a critical factor in the suppression of cancer cell proliferation. As curcumin administration increases the CRC cell apoptosis as depicted in Figure 3, we speculate that curcumin administration may facilitate the expression of p53 molecules in CRC tissue. In order to test the hypothesis, we observed the p53 expression in cancer tissues by immunohistochemistry. The results showed that there were certain amount of $p53^+$ cells present in CRC tissue before treatment in both curcumin and vehicle groups. Treatment with curcumin markedly increased the p53 expression in CRC tissue (Figure 4).

Levels of Bax increased and levels of Bcl-2 decreased in cancer cells after curcumin treatment

We observed that the curcumin treatment increased p53 expression (Figure 4) and apoptotic cells in CRC tissue (Figure 3); the data implicate that curcumin administration initiates apoptotic pathway in CRC tissue. In order to

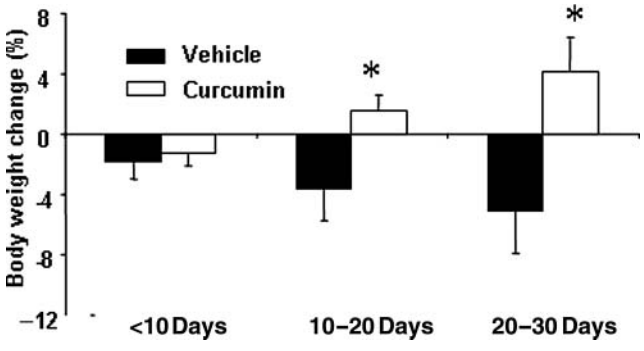

Figure 1. Body weight change after treatment with curcumin. Sixty-three CRC patients were treated with curcumin (curcumin group); another 63 CRC patients took vehicle (vehicle group) ahead of surgery. The treatment lasted for 10–30 days. Each group consisted of 21 patients. Body weight was recorded for each patient before and after the curcumin treatment. Bars indicate the change rate (%) of body weight. $^*p < .05$, compared with the data obtained before treatment and after treatment from the same group.

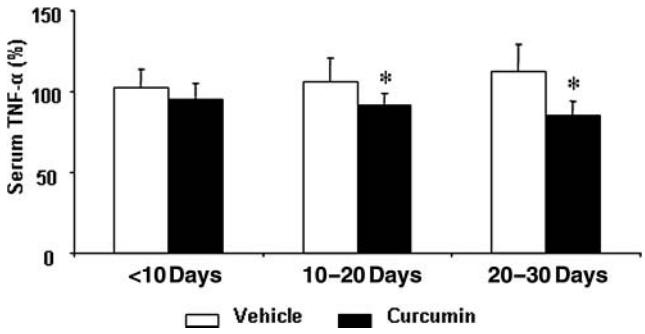

Figure 2. Effect of curcumin administration on serum levels of TNF-α in CRC patients. CRC patients were treated with curcumin or vehicle as described in Figure 1. The sera were collected from the patients before and after the treatment. Levels of TNF-α in the sera were assessed by ELISA. Bars indicate the percentage of serum TNF-α change after curcumin treatment (compared to the serum TNF-α level before treatment). $^*p < .05$, compared with the same group before treatment.

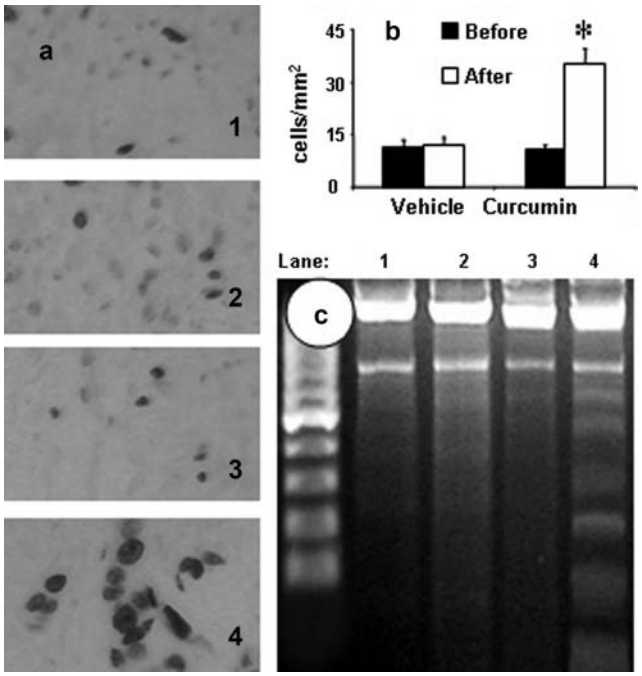

Figure 3. Administration with curcumin induces CRC cell apoptosis. CRC tissue was collected before and after treatment with curcumin or vehicle in the CRC patients as described in Figure 1. (a) Cryosections were prepared from CRC tissue and stained by TUNEL. Apoptotic cells were stained in dark. (a1) and (a2): CRC tissue was collected from CRC patients treated with vehicle. (a3) and (a4): CRC tissue was collected from CRC patients treated with curcumin. (a1) and (a3): samples were obtained before treatment. (a2) and (a4): samples were obtained from surgically removed CRC tissue. (b) Bars indicate the apoptotic cells in panel (a). Cells were counted from 30 high power fields (400×) for each sample. Slides were coded. The observer was not aware of the codes to avoid the observer bias. Data are presented as mean ± SD. $^{*}p < .05$, compared with samples collected before treatment. (c) DNA was extracted from CRC specimens and separated by agarose gel. Lanes 1 and 2: from vehicle group. Lanes 3 and 4: from curcumin group. Lanes 1 and 3: from biopsies at diagnosis (before treatment). Lanes 2 and 4: from surgically removed tumor (after treatment).

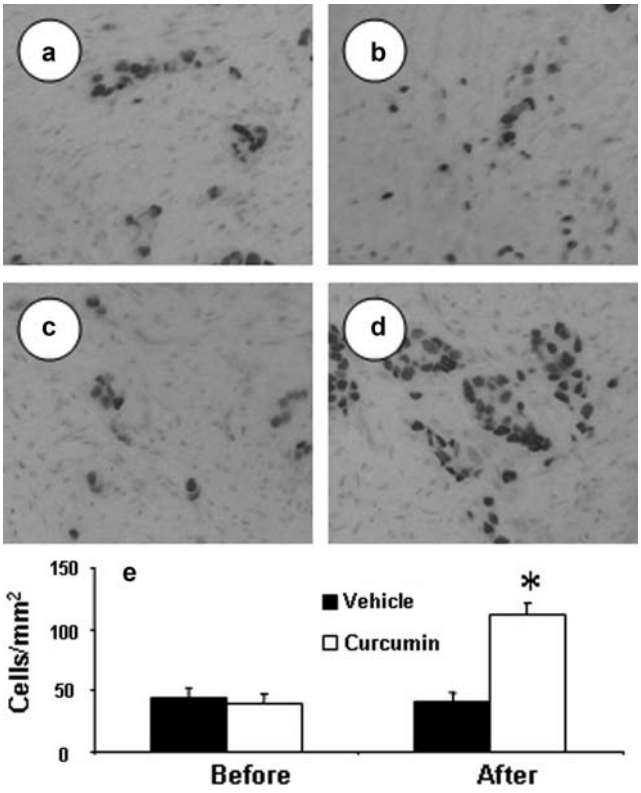

Figure 4. Curcumin increases p53 expression in cancer cells. CRC tissue was obtained from CRC patients treated with curcumin or vehicle as described in Figure 1. The collected CRC tissue was cut in cryosections and stained with anti-p53 antibody by immunohistochemistry. (a)–(d): representative images show p53 positive cells (the darkly stained cells) in CRC tissue. (a) and (b): vehicle group. (c) and (d): curcumin group. (a) and (c): before treatment. (b) and (d): after treatment. (e) Bars indicate p53 positive cells in CRC tissue. $^{*}p < .05$, compared with the data within the same group before and after treatment.

confirm the results, we analyzed the expression of two important molecules, Bax and Bcl-2, in apoptotic pathway by immune blotting. Indeed, the results showed that treatment with curcumin increased the Bax expression and inhibited the Bcl-2 expression in CRC tissues (Figure 5).

DISCUSSION

The present data indicate that treatment with curcumin leads to body weight gain in CRC patients. We have also observed that curcumin administration increases the p53 expression in CRC cells, increases CRC tissue DNA fragmentation, and modulates the Bax and Bcl-2 expressions in CRC.

Weight loss is a general sign in patients with cancer (11–13). It is one of the major symptoms of cachexia; the latter includes several symptoms, such as weight loss, waste of muscles, loss of appetite, and general debility that can occur during chronic disease, especially in cancer (11–13). Most of cachexia is caused by the diminished consumption of nutrients rather than by a hypermetabolic state (14). It results from cancer cells' nutrition consumption and noxious cytokine release that have toxic effects on the body (15). Taking the advantage of the waiting period before surgery, we treated a group of CRC patients with curcumin that resulted in

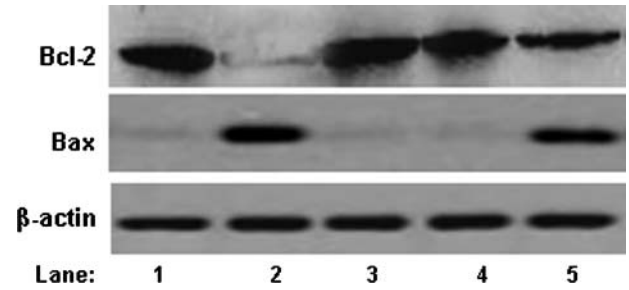

Figure 5. Levels of Bax and Bcl-2 in cancer tissue. CRC specimens were obtained before and after treatment with curcumin as described in Figure 1. Protein was extracted from CRC specimens and separated by PAGE gel. Lanes 1 and 2: from curcumin group. Lanes 3 and 4: from vehicle group. Lanes 1 and 3: samples were obtained before treatment. Lanes 2 and 4: samples were obtained after treatment. Lane 5: from control tissue (the marginal non-cancerous sample of surgically removed tissue).

favorable consequences, namely, improvement in weight loss in CRC patients. The data implicate that the curcumin treatment could improve the general health of CRC patients. Currently there are not much satisfactory remedies available for the treatment of cachexia in cancer patients; the present data provide a novel method to improve the cachexia.

The mechanism of cancer cachexia is not fully understood yet. The persistent inflammatory response of the host, in conjunction with inappropriate production and release of cytokines, such as TNF-α, IL-1, and IL-6, is essential to disease pathogenesis (16). Our study provides supportive data to the existing data by showing that high serum levels of TNF-α were detected in patients with colorectal cancer. The novel aspect of the present study is that we found that after the curcumin treatment, the serum levels of TNF-α were markedly suppressed. This should be responsible for the improvement of cachexia in cancer patients because the suppression of serum levels of TNF-α is negatively correlated with the body weight gain. Other substance such as *Sophora flavescens* Ait. (Kushen) also has similar effect as reported previously (17).

Cumulative reports from animal studies indicate that curcumin has the inducing effect on cancer cell apoptosis (18). Bearing this notion in mind, we examined the DNA fragmentation and the frequency of apoptotic cells in CRC tissue. In line with those animal studies (18), we found that curcumin administration in CRC patients also induced cancer cell apoptosis, which could reduce the cancer cells in tumor. The reduction of cancer cells might, at least partially, contribute to the improvement of weight loss and the reduction of serum levels of TNF-α as we observed in the present study.

It is proposed that p53 is one of the key tumor suppressors, which modulates both mitochondria-dependent and mitochondria-independent apoptotic pathways and functions as an apoptosis inducer in response to various stressors, including DNA damage, hypoxia, and oncogenetic activation (19). As we found that after the curcumin treatment the rate of apoptotic cells increased significantly in colorectal cancer, we may consider that the apoptosis in cancer cells is induced by curcumin administration, which is in line with previous experimental studies (20).

There are several key molecules, including Bax and Bcl-2, that have been identified playing a crucial role in the signal transduction pathway of apoptosis. We also examined the effect of curcumin administration in modulating the expression of Bax and Bcl-2 in CRC tissue. The results confirmed our hypothesis that before the curcumin treatment, low levels of Bax and high levels of Bcl-2 expressions were observed in cancer tissue as compared with normal controls. The curcumin administration showed a favorable effect on the modulation of Bax and Bcl-2 expressions in cancer tissues.

In summary, the present study provides evidence that curcumin administration improves weight loss, reduces serum levels of TNF-α, increases cancer cell apoptosis, upregulates p53 molecules, and modulates apoptosis-related Bax and Bcl-2 molecules in cancer cells. Thus, the curcumin administration can be a supplemental remedy for the treatment of cancer.

DECLARATION OF INTEREST

The authors report no conflict of interest. The authors alone are responsible for the content and writing of this paper.

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