Demethoxycurcumin Inhibits *In Vivo* Growth of Xenograft Tumors of Human Cervical Cancer Cells

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Abstract. *Background/Aim: Demethoxycurcumin (DMC), a derivate of curcumin from natural plants, exerts antitumor effects on various human cancer cells in vitro and in vivo. Nevertheless, no reports have disclosed whether DMC can affect the growth of human cervical cancer cells in vivo. Therefore we investigated the antitumor effects of DMC on a HeLa cell xenograft model in nude mice in this study. Materials and Methods: Twenty-four nude mice were subcutaneously injected with HeLa cells. All mice were randomly divided into control, low-dose DMC (30 mg/kg), and high-dose DMC (50 mg/kg) groups and individual mice were treated intraperitoneally accordingly every 2 days. Results: DMC significantly reduced tumor weights and volumes of HeLa cell xenografts in mice, indicating the suppression of growth of xenograft tumors. Conclusion:*

These effects and findings might provide evidence for investigating the potential use of DMC as an anti-cervical cancer drug in the future.

Cervical cancer is the seventh most common cancer globally and the fourth most common type of cancer in women (1, 2). In 2018, there were an estimated 567,000 new cases and 311,000 deaths from cervical cancer globally (3). About 80% of cervical cancer arises in developing countries (3). Among females, cervical cancer is the second most commonly diagnosed cancer and the third leading cause of cancer death in less developed countries (4, 5). In Taiwan, the 2017 annual report of the Ministry of Health and Welfare indicated that approximately four individuals per 100,000 die annually with cervical cancer, and it was the eighth cause of cancer-related death (6). Currently, surgery, radiotherapy, chemotherapy, and immunotherapy are four main treatment options for cervical cancer; however, the treatment of patients with cervical cancer depends on the cancer stage and tumor location according to diagnosis and characteristics of the patients (7, 8).

The incidence of cervical cancer is increasing worldwide. Chemotherapy is one of the strategies for treatment and it has been demonstrated to trigger effective response and improves overall survival in many patients. However, cancer may develop resistance to chemotherapies and lead to treatment failure (9, 10). Other side-effects of the current chemotherapy treatment of patients with cervical cancer are fatigue, nausea, vomiting, and diarrhea (11). Therefore, many studies have focused on finding and seeking new compounds from natural products for treating cervical cancer.
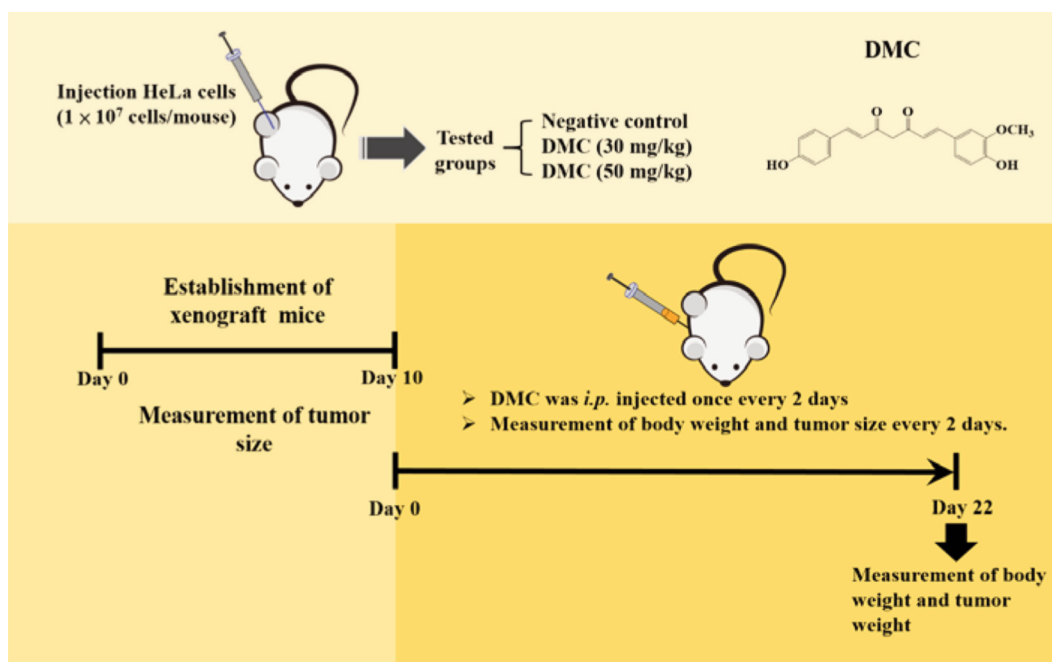
Figure 1. *The experimental design for the demethoxycurcumin (DMC) treatments in human cervical HeLa cell-bearing mice. Each nude mouse was injected with 1×10^7 HeLa cells. Animals were randomly divided into three different treatment groups (n=8 for each group; control (NC) and 30 mg/kg and 50 mg/kg of DMC) when the tumor volume reached 100-120 mm³ in each mouse. DMC was administered every 2 days by intraperitoneal injection. All mice were sacrificed at 22 days after treatment.*

Curcuminoids are found in *Curcuma longa* Linn. They are polyphenol compounds and include curcumin, demethoxycurcumin (DMC), and bisdemethoxycurcumin (BDMC) present at a ratio of 77:17:3, respectively (12). Curcumin has been widely used as natural food additive in Asian countries, especially in India and China (13, 14). DMC, a derivative of curcumin, is more chemically stable than curcumin (15) and induced cell apoptosis GBM 8401 human malignant glioma cells *via* mitochondria- and caspase-dependent pathways (16). DMC significantly inhibited cell proliferation and metastasis activity, including cell migration and invasion in PC-3 human prostate cancer cells *in vitro* (17). It also induced cell apoptosis and DNA damage in NCI-H460 human lung cancer cells (18). DMC reduced cell viability and induced cell cycle arrest (G_2/M) in A431 skin cancer cells and HaCaT human keratinocyte cells (19). In addition, DMC showed higher growth inhibition of glioblastoma stem cells than did temozolomide *in vivo* (20). Recently, we found that DMC induced cell apoptosis of human oral cancer cells *via* ROS- and mitochondrial-dependent pathway and cell autophagy (21). However, as far as we are aware, there is no report to show the effect of DMC on growth of cervical cancer cells *in vivo*. Therefore, we investigated the effects of DMC suppressed tumor growth of human cervical cancer HeLa cell xenograft in nude mice and results indicated DMC significantly inhibited tumor growth *in vivo*.

Materials and Methods

Test chemicals, reagents and culture medium. DMC, dimethyl sulfoxide (DMSO), and trypan blue were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Dulbecco's modified Eagle's medium, fetal bovine serum (FBS), L-glutamine, and antibiotics (penicillin and streptomycin) were purchased from GIBCO®/ Invitrogen Life Technologies (Grand Island, NY, USA). DMC was dissolved in DMSO before use.

Cell culture. HeLa human cervical cancer cell line was purchased from the Food Industry Research and Development Institute (Hsinchu, Taiwan, R.O.C.) and cultured based on the supplier's instructions. Cells were cultured in DMEM containing 10% FBS, 2 mM L-glutamine, 100 Units/ml penicillin, and 100 μg/ml streptomycin. Cells were grown in a 75 cm² culture flasks under a humidified 5% CO_2 and 95% air at 37˚C at 1 atmosphere as described previously (22).

Animals and treatments. Twenty-four athymic male mice (CAnN.Cg-*Foxn1ⁿᵘ*/CrlNarl nude mice; six-week-old) were obtained from the National Laboratory Animal Center (Taipei, Taiwan, R.O.C.). All animals were used in this study according to the National Institutes of Health Guidelines for Animal Research. All animals were housed at the Animal Center of China Medical University (Taichung, Taiwan, ROC) and approved by the Institutional Animal Care and Use Committee of China Medical University (number: 106-asia-13). HeLa cells (1×10^7) were resuspended in 50 μl of serum-free DMEM and then mixed with 50 μl of Matrigel. The cell mixture was subcutaneously inoculated into
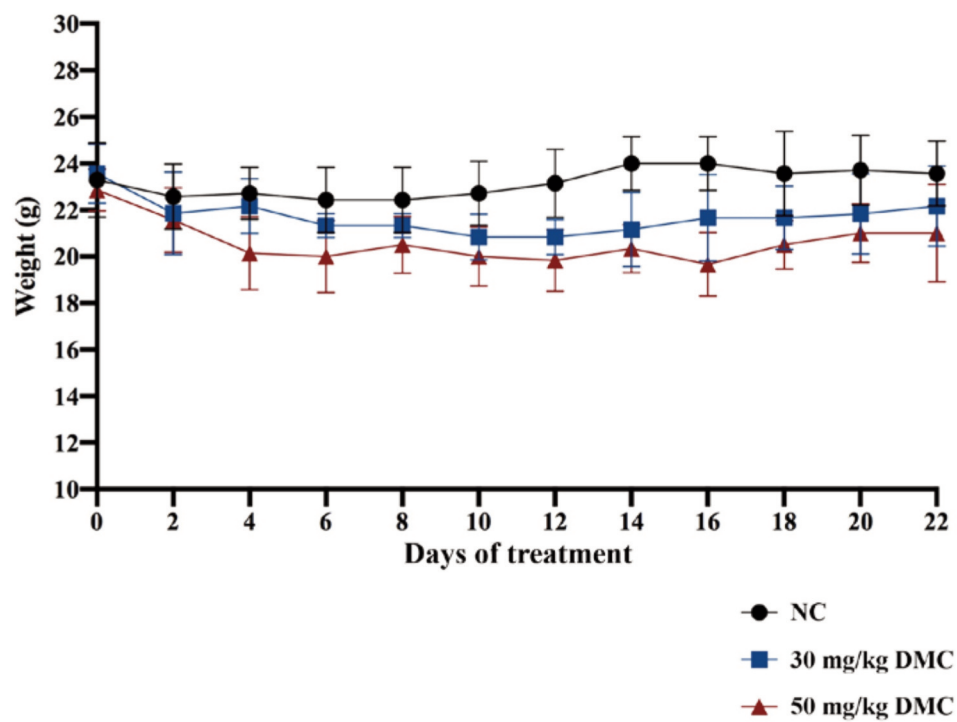
Figure 2. *The effects of demethoxycurcumin (DMC) on the body weights of xenografts in HeLa cell-bearing mice. The body weights of control (NC) and DMC-treated (30 mg/kg and 50 mg/kg) mice were measured and recorded every 2 days for a total of 22 days and presented. The data are expressed as the mean±S.D.*
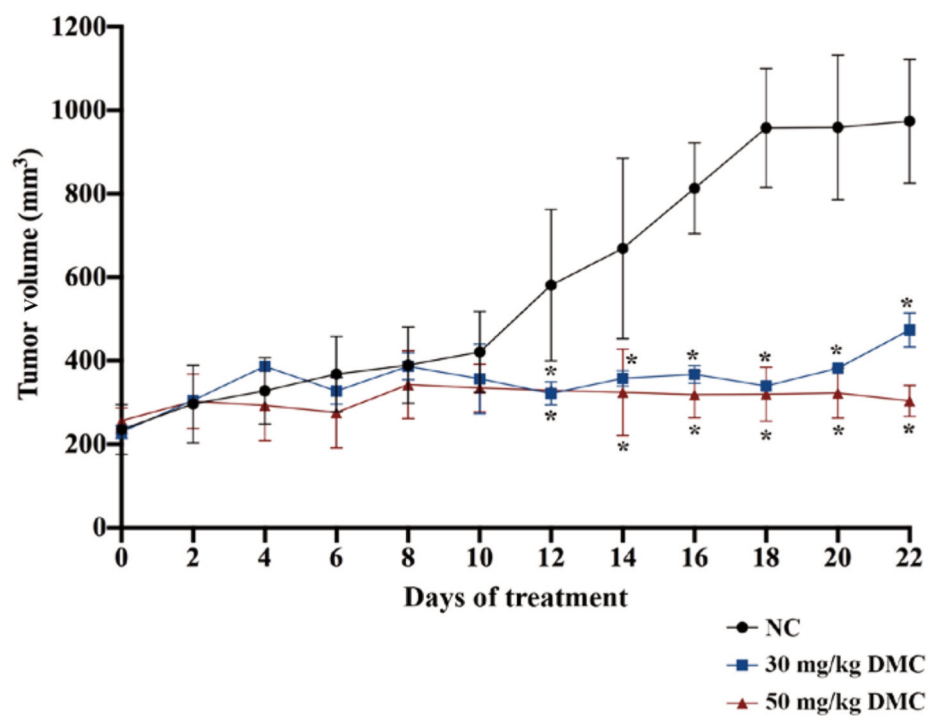

Figure 3. *Antitumor evaluation of demethoxycurcumin (DMC) in xenografts in HeLa cell-bearing mice. Tumor volume was individually measured in control (NC) and DMC-treated (30 mg/kg and 50 mg/kg) mice every 2 days. *Significantly different from the control at p<0.05. The data are expressed as the mean±S.D.*
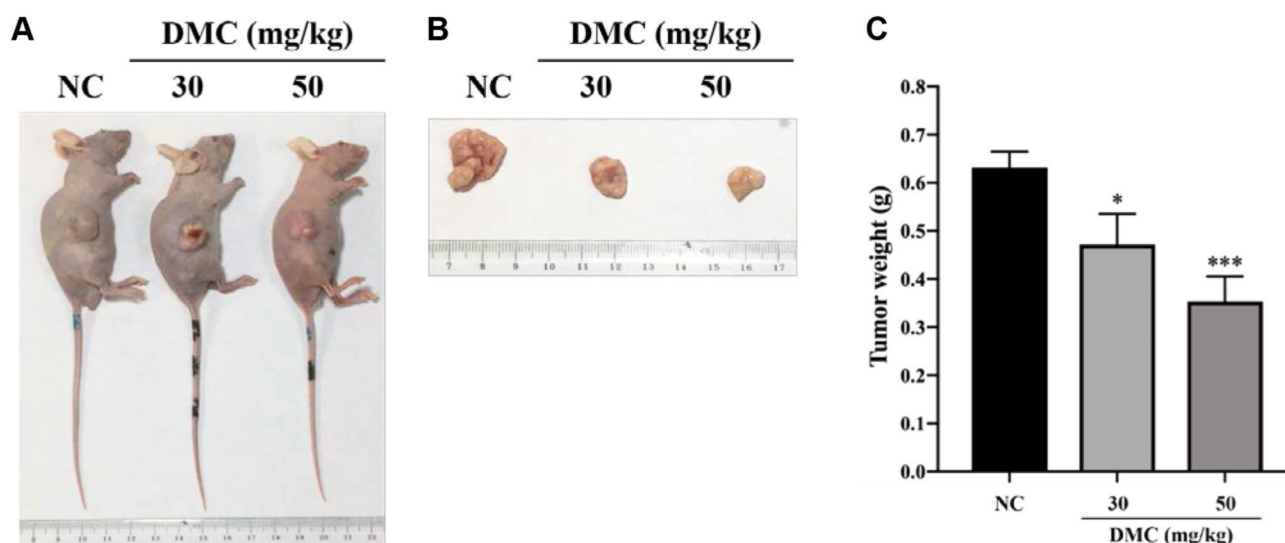
Figure 4. *Antitumor evaluation of demethoxycurcumin (DMC) in xenografts in HeLa cell-bearing mice. A: The tumor growth of each mouse was monitored and representative tumors on control (NC) and DMC-treated (30 mg/kg and 50 mg/kg) mice from the three groups after 22 days of treatment are presented. B: Representative tumors from three groups are shown. C: DMC at both doses (30 and 50 mg/kg) significantly suppressed tumor growth when compared to the control group. Significantly different from the control at *p<0.05 and ***p<0.001. The data are expressed as the mean±S.D.*

the right hind flank of nude mice. Tumor growth in each mouse was monitored by a digital caliper and calculated with the equation: tumor volume=0.523×length×width^2 (23).

All animals were randomly divided into three different treatment groups (control, 30 mg/kg DMC, and 50 mg/kg DMC; n=8 for each group) when the tumor volume reached 100-120 mm^3 in each mouse. The control group animals were treated with 45 μl phosphate-buffered solution (PBS) plus 5 μl DMSO by intraperitoneal (*i.p.*) injection every 2 days for 22 days. Experimental groups were treated with 30 mg/kg or 50 mg/kg of DMC by *i.p.* injection every 2 days for 22 days. The body weight and tumor volume of each mouse from each group were measured after treatment. At the end of treatment, all mice were sacrificed for isolating the tumor, as described previously (24). Individual tumor weights of mice from three groups were recorded every 2 days. The flowchart of the experimental design is displayed in Figure 1.

Statistical analysis. All data from the three groups are presented as the mean±standard error. For the comparison between DMC-treated and control groups, one-way ANOVA with Newman-Keuls multi-comparison test was used. Differences between the experimental groups and the control at *p*<0.05 or less were recognized to be significant.

Results

DMC affected the body weight of animals with HeLa cell xenograft. As shown in Figure 2, there was no significant difference in body weight among the three groups (control, 30 mg/kg and 50 mg/kg of DMC), which reflected no signs of acute or delayed toxicity of DMC in the examined mice.

DMC inhibited tumor xenograft growth of HeLa cells. After treatment for 12 days, DMC significantly diminished tumor

volume when compared to the control (0.1% DMSO/PBS) group. Tumor volume was significantIy lower after 22-day DMC treatment compared to the control (Figure 3).

After treatment, all mice from each group were sacrificed and representative mice were photographed and are shown in Figure 4A. Subsequently, the individual tumor was collected from each mouse and representative tumors are presented in Figure 4B. The final tumor weight was measured and recorded in Figure 4C.

Both doses of DMC (30 and 50 mg/kg) significantly reduced the tumor volume in comparison with the control group, and DMC at a high dose (50 mg/kg) resulted in greater reduction of tumor volume than did the low dose (30 mg/kg) (Figure 4B). Similarity, both DMC treatments also significantly diminished the tumor weights in comparison with the control group, with high-dose DMC more greatly reducing the tumor weight than the low dose (Figure 4C).

Discussion

Currently, the preventative and therapeutic protocols for cervical cancer depended upon the stage of cancer, and human papillomavirus vaccines and Pap screening have been shown to be useful in preventing or detecting pre-cancerous lesions, respectively (25, 26). Typical clinical treatment of patients with cervical cancer includes surgery, radiation, and cisplatin-based chemotherapy (27). Unfortunately, the development of drug resistance is common. Numerous studies have attempted to focus on finding new approaches

or new compounds from natural products to reduce drug toxicity and overcome resistance.

In the present study, we found that DMC at 30 and 50 mg/kg significantly reduced tumor volume (Figure 3) and tumor weight (Figure 4C) without adverse effects on body weight of mice. This supports findings of Ni *et al*. for the effects of another derivative of curcumin on subcutaneous xenograft tumors in human cervical HeLa cell-bearing mice *in vivo* (28, 29). These findings are also in agreement with previous study on SAS human oral cancer cell xenografts in nude mice (29). A decrease of body weight is an important marker in evaluating whether or not agents induce cytotoxic effects *in vivo*.

DMC is attractive for its antitumor effects on different mouse models of human cancer. The therapeutic potential of DMC was investigated in A549 human lung cancer cell xenograft mouse model (30). DMC inhibited tumor growth more effectively than temozolomide in an orthotopic glioblastoma model (31). Further study of co-treatment of DMC and temozolomide showed that DMC at a low dose enhanced the sensitivity of glioma cells to temozolomide *in vitro* but that at high dose presented greater effects on glioblastoma cells *in vitro* and *in vivo* compared with temozolomide treatment alone (32).

We found that DMC significantly suppressed the size and weight tumor from HeLa cell xenografts in nude mice *in vivo* and these findings are also consistent with reports in xenograft mouse models of glioma and lung cancer. This study is the first to show that DMC has anticervical tumor potential in an animal model *in vivo*.

Previous study by our groups has indicated that DMC had potent anti-metastasis effects on HeLa cells *in vitro* (4). Recently, we also demonstrated that gefitinib combined with DMC treatment in nude mice bearing SAS human oral cancer cell xenografts significantly reduced the tumor weight and volume, and did not affect the total body weight (29). Co-treatment with DMC and temozolomide was shown to have synergistic activity in the induction of cell apoptosis and the inhibition of cell proliferation in glioblastoma multiforme cells (32). Investigations regarding the molecular mechanism of DMC are needed.

In conclusion, DMC significantly diminished the tumor weights, size, and volumes of HeLa xenograft tumors *in vivo*. These results indicate that DMC can be considered as a potential drug for cervical cancer.

Conflicts of Interest

The Authors confirm that there are no conflicts of interest.

Authors' Contributions

Study conception and design: FSC, HYC and SFP; Acquisition of data: FSC, JYH, JYK, WNH, SYS and HYT; Analysis and interpretation of data: FSC, JCL, YCC and WWH; Drafting of manuscript: FSC, HYC and SFP; Critical revision: FSC, HYC and SFP All Authors discussed the results and commented on the article.

Acknowledgements

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