Dimethyl fumarate suppresses hepatocellular carcinoma progression via activating SOCS3/JAK1/STAT3 signaling pathway

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Abstract: Dimethyl fumarate (DMF) is generally used to treat psoriasis and multiple sclerosis. In the present study, we aimed to investigate the effects of DMF on hepatocellular carcinoma progression and its mechanism of action. In vitro, cell viability was examined using CCK-8 assay; cell cycle was analyzed by flow cytometry; angiogenesis was detected using tube formation assay; and autophagic flux assay results were examined using fluorescence microscopy. We also used western blotting to explore the potential mechanisms. In vivo, tumor xenograft experiment was performed with nude mice, and liver function, renal function, and routine blood counts were assessed using biochemical tests. Dimethyl fumarate inhibited tumor growth and angiogenesis in hepatocellular carcinoma, both in vitro and in vivo. Dimethyl fumarate decreased autophagy in hepatocellular carcinoma cells. Treatment with DMF activated SOCS3, which led to repression of JAK1 and STAT3 phosphorylation. DMF inhibited cell proliferation, angiogenesis, and autophagy via activation of the SOCS3/JAK1/STAT3 signaling pathway. This finding may provide a novel approach for the treatment of hepatocellular carcinoma.

Keywords: Dimethyl fumarate (DMF), SOCS3/JAK1/STAT3 pathway, cell proliferation, autophagy, hepatocellular carcinoma

Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignancies worldwide [1], Sorafenib is the standard treatment for patients with advanced HCC; however, the outcomes remain poor [2]. Several systemic therapeutic drugs against HCC have been tested in an attempt to overcome the poor anti-tumor effects and toxicity but satisfactory results have not been observed, for various reasons [3].

Dimethyl fumarate (DMF) is an inexpensive compound commonly used in industrial chemistry. Initially, it was used to treat psoriasis [4]. Recently, DMF was approved by the US Food and Drug Administration and the European Medicines Agency for the treatment of multiple sclerosis (MS) [5]. Long-term intake of DMF does not cause serious adverse effects [5-7]. Dimethyl fumarate exerts anti-inflammatory effects by inhibiting the expression of inflammatory cytokines such as NOS, IL-1β, and IL-6 in glial cells [8, 9]. Furthermore, DMF has also been found to exhibit anti-tumor effects. It not only inhibits melanoma growth and metastasis but also induces colon cancer apoptosis and necroptosis [10, 11]. However, the effects of DMF on HCC and its mechanism of action remain unknown.

The Janus kinase/signal transducer and activator of transcription (JAK/STAT) pathway is constitutively activated in cancers and is involved in a variety of cellular functions such as differentiation, survival, proliferation as well as apoptosis [12-14]. STAT3 protein plays a key role in tumorigenesis, tumor cell proliferation, survival, and invasion [15]. STAT3 transcriptional activity is primarily activated by phosphorylation of a single tyrosine residue Tyr705. Tyrosine phosphorylation of STAT3 can be catalyzed by the
kinase JAK [16]. Once activated, STAT3 regulates the transcription of many target genes, such as the angiogenesis-related gene VEGF [17] and the autophagy-related genes BECN1 [16] and ATG5 [18]. Suppressors of cytokine signaling protein 3 (SOCS3) was reported to negatively regulate the JAK/STAT pathway [19] through attenuation of JAK activity. In addition, recent studies have demonstrated universal activation of the JAK/STAT pathway in human HCC [20], suggesting that JAK/STAT inhibitors could be used for treating HCC [21].

In the present study, we demonstrated that DMF suppressed hepatocellular carcinoma progression via the SOCS3/JAK1/STAT3 signaling pathway, which may provide a novel solution for the treatment of liver cancer.

Materials and methods

Cell culture

Two HCC cell lines (Huh-7 and HCC-LM3), a hepatoblastoma cell line (HepG2), and a human umbilical vein endothelial cell (HUVEC) line were used in the present study. The cells were purchased from Cell Bank of Type Culture Collection of Chinese Academy of Sciences, Shanghai Institute of Cell Biology, Chinese Academy of Sciences, and were cultivated as described by the suppliers. Huh7, HCC-LM3 and HepG2 cells were cultured in modified Eagle’s medium (MEM; Biological Industries, CA, USA) supplemented with 10% fetal bovine serum, and HUVECs were cultured in endothelial cell growth medium at 37°C and 5% CO₂.

Reagents

DMF (CAS: D106460) was purchased from Aladdin Industrial Corporation (Shanghai, China). Rapamycin (CAS: S1039) was purchased from Selleck Chemicals (Shanghai, China). DMSO (CAS: 67-68-5, D8418) was obtained from Sigma-Aldrich, Inc. (Darmstadt, Germany). Methyl cellulose (CAS: 9004-67-5) was obtained from Sangon (Shanghai, China).

Antibodies

STAT3 (#30835), phospho-STAT3 (Tyr705)(#9145), JAK1 (#50996), phospho-JAK1 (#74129), Atg5 (#9980), Atg7 (#8558), LC3B (#3868), Beclin1(#3495), and phospho-VEGF receptor 2 (#3770) antibodies were purchased from Cell Signaling Technology (USA). VEGFA (Cat. No. 66828-1-lg), CKD2 (Biotech Cat. No. 10122-1-AP), (Cat. No. 11026-1-AP), P21 (Cat. No. 10-355-1-AP), cyclin D1 (Cat. No. 60186-1-lg), and Ki67 (Cat. No. 27309-1-AP) antibodies were obtained from Proteintech (Rosemont, IL, USA). Anti-CD31 antibody (#GB1306) was obtained from Servicebio (China). Bcl-XL antibody (#db-225) was obtained from Bio-Technology Co., Ltd. (Hangzhou, China), c-Myc (ab32072) and Anti-AKT1 (phospho S473) (ab81283) antibodies were obtained from Abcam (USA).

Immunohistochemical analysis

Tumor tissues from mice were sliced into 4 µm thick sections using a thin semi-automatic slicer. All sections were deparaffinized in xylene and rehydrated in a series of graded alcohol dilutions. Antigen retrieval was performed by heating in a microwave oven. The sections were then incubated with 3% H₂O₂ for 10 min and then incubated with 10% normal goat serum for 15 min at room temperature to block endogenous peroxidase as well as non-specific antigen binding. The samples were then incubated with primary antibodies. Tissue sections were immunostained overnight at 4°C with the following antibodies: anti-Ki67 antibody (1:1000 dilution; #27309-1-AP, Proteintech, Rosemont, IL, USA), anti-LC3B antibody (1:200 dilution; #3868, Cell Signaling Technology, USA) and anti-CD31 antibody (1:200 dilution; GB1306, Servicebio, China). After washing thrice with phosphate buffered saline (PBS), the sections were incubated with horseradish peroxidase-labeled secondary antibody (1:2000 dilution) for 30 min at room temperature. Finally, 3,3-diaminobenzidine was used to visualize the signal development. The sections were then counterstained with hematoxylin. Finally, the sections were photographed under an inverted microscope.

CCK-8 assay

Cell vitality was determined with the cell counting kit-8 (CCK-8) according to the manufacturer’s protocol (DOJ INDO Laboratories, Kumamoto, Japan). In brief, cells were seeded into 96-well plates at a density of 5000 cells per well. 12 h later, cells were treated with different concentrations of DMF in a humidified incubator for 24, 48, or 72 h. After removing the supernatant by vacuum, 100 µL serum-free medium and 10 µL of CCK-8 were premixed and added to each well, followed by incubation for 1 h. Absorbance was measured at 450 nm using a microplate reader (BioTek, USA). Each assay was performed in triplicate.
Colony formation assay

Cells were seeded in 6-well plates at an equal density (1000 cells per well) and were treated with different concentrations of DMF. Fresh medium was replaced every 4 days. Cells were maintained in culture for another 10 days; the medium was removed and cells were fixed with methanol for 10 min and then stained with crystal violet. The total number of colonies in each dish were counted and recorded from three independent experiments.

Tube formation assay

Matrigel (40 µL) was uniformly coated on 96-well plates, and HUVECs were seeded at a density of 2 × 10^4 cells/well in Matrigel-coated 96-well plates containing 50, 100, or 200 µM DMF. After cells were cultured for 6-8 h, the capillary-like structures of HUVECs were photographed under an inverted microscope. The branch points of formed tubes, which represent the extent of angiogenesis in vitro, were scanned and quantified at 100 × magnification.

Flow cytometry analysis

Cells (1.5 × 10^5 per well) were seeded into 6-well plates and treated with different DMF concentrations for 48 h. Cells were harvested and fixed with pre-cooled 75% ethanol at -20°C for 24 h, then resuspended in 300 µL of DNA-Prep stain (Beckman Coulter, USA), and incubated for 30 min at room temperature in the dark. Suspended cells were analyzed by flow cytometry (BD FACSCalibur, USA).

Autophagic flux assay

Huh7 cells (2 × 10^4) were seeded in 6-well plates and incubated overnight. mCherry-GFP-LC3 adenovirus (HANBIO, Shanghai, China) was transfected with 5 µg/mL polybrene. The transfection mixture was replaced with 10% fetal bovine serum containing MEM overnight and cultured for an additional 24 h. Cells were then treated with rapamycin or rapamycin in combination with DMF for 24 h. Green (GFP) and red (mCherry) fluorescence was observed under a fluorescence microscope.

Western blot assay

After the cells were treated with different concentrations of DMF, cells were harvested and lysed in RIPA buffer, and supernatants were collected and stored at -20°C after centrifugation at 12000 × g for 15 min. Protein concentrations were determined by Bradford assays (Bio-Rad, USA). After denaturation, proteins were separated by gel electrophoresis using 10% or 12% SDS-polyacrylamide gels and then transferred to a PVDF membrane for 1 h followed by blocking in 5% non-fat milk. Membranes were washed once with TBST (0.1% Tween 20) and then incubated overnight at 4°C with the relevant antibodies (1:1000 dilution). The next day, membranes were washed three times with TBST for 10 min each time and then incubated with secondary antibody (goat anti-rabbit IgG, 1:2000 dilution) for 1 h at room temperature. After a washing procedure as in the previous step, ECL liquid was added, and samples were placed in a dark room to allow the reaction to proceed. GAPDH (1:1000 dilution) was used as a positive control.

Tumor xenograft experiments

All experimental protocols were approved by the animal experimental ethics committee of First Affiliated Hospital of Zhejiang University School of Medicine. The procedures were carried out in accordance with the approved guidelines. HCC-LM3 cells (3 × 10^6) were resuspended in 50 µL phosphate buffered saline and injected subcutaneously into the flanks of immunodeficient mice. Tumor formation was monitored by palpation, and when tumors were detected, mice were randomized into control or DMF treatment groups. Mice were injected daily with vehicle (0.8% methylcellulose) or DMF (30 mg/kg suspended in 0.8% methylcellulose). Tumor size was measured daily using electronic calipers and calculated using the following equation: Volume (mm^3) = width^2 (mm^2) × length (mm)/2. Tumor growth was monitored until the total tumor burden reached humane endpoint criteria. All tumors were harvested for immunostaining and western blotting assays.

Biochemical tests

Each blood sample (1 mL) was divided into two Eppendorf tubes containing heparin, and one of the tubes was centrifuged at 3000 × g for 5 min to obtain serum. Typical biochemical markers of hepatocyte lysis, such as circulating alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase...
DMF suppresses HCC progression

Figure 1. DMF suppressed cell proliferation of HCC. A-C. Cell Counting Kit-8 assay was used to detect the proliferation of HCC-LM3, Huh7 and HepG2 cells after treated with DMF. D and E. Colony formation assay evaluated the growth of HCC-LM3, Huh7 and HepG2 cells treating with 50 μmol and 100 μmol DMF. Values are presented as the mean ± SD, every experiment was repeated three times. *P < 0.05, **P < 0.01 and ***P < 0.001.

Results

Dimethyl fumarate inhibits cell proliferation in HCC

Previous studies have shown that DMF is capable of inhibiting melanoma and colon cancer cell growth [10, 11]. In the present study, we analyzed whether DMF treatment also exhibits cytotoxicity against HCC cells. The CCK-8 assay was performed to investigate the effect of DMF on proliferation in HCC cells (HCC-LM3, Huh7, and HepG2). As shown in Figure 1A-C, cell via-

Statistical analysis

SPSS 18.0 software was used for statistical analysis. Quantitative data were expressed as mean ± SD. Means were compared using Student’s t-test. P values < 0.05 were considered statistically significant.

(ALP), and those of renal function, such as creatinine (CR), blood urea nitrogen (BUN), and uric acid (UA), were analyzed. The other blood sample tube was used to detect the red blood cell, white blood cell, and platelet counts.

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Dimethyl fumarate inhibits angiogenesis of HUVECs

To analyze whether DMF exhibits distinct anti-angiogenic effects in addition to its known anti-tumor function, we used tube formation assays to investigate the effect of DMF on the ability of HUVECs to form capillary-like structures. Dimethyl fumarate inhibited angiogenesis in HUVECs in a dose-dependent manner, as determined by the tube formation assay (Figure 3A and 3B).

To elucidate the molecular mechanism underlying inhibition of angiogenesis by DMF, western blot assay was used to analyze the expression of p-VEGFR2 and VEGFA in HUVECs after DMF treatment. After DMF treatment, p-VEGFR2 and VEGFA were found to be downregulated in HUVECs. Thus, the anti-angiogenic effects of DMF corresponded with suppression of p-VEGFR2 and VEGFA protein expression (Figure 3C).

Dimethyl fumarate activates SOCS3 and inhibits JAK1/STAT3 signaling pathway

In addition to affecting proliferation, DMF also affects apoptosis of HCC cells. Our results

Figure 2. DMF arrested HCC cell cycle at G1 phase. A and B. Cell cycle arrest was examined by Flow cytometry for the treatment of HCC cells with DMF. Values are presented as the mean ± SDs, every experiment was repeated three times. **P < 0.01 compared with DMSO group. C and D. HCC-LM3 cells and HepG2 cells were treated by DMF. Cell cycle associated protein p21, CDK2 and cyclinD1 were examined by western blot.

Dimethyl fumarate arrests cell cycle at G1 phase

Based on the results that DMF alters HCC cell proliferation, we assumed that DMF treatment may affect the cell cycle. Therefore, flow cytometry was performed. We found a significant increase in G1 phase arrest in the DMF group, accompanied by a significant decrease in the S phase (Figure 2A and 2B) (P < 0.05).

To elucidate the molecular mechanism underlying the changes in cell cycle, we performed western blot and found that after DMF treatment, cell cycle-associated protein p21 was upregulated and CDK2 and cyclin D1 were downregulated in HCC-LM3 and HepG2 cells (Figure 2C and 2D).

bility of HCC-LM3, Huh7, and HepG2 was significantly inhibited by different concentrations of DMF in a dose-dependent manner (P < 0.05 compared with the DMSO group). Results of the colony formation assay also showed that DMF significantly suppressed the growth of HCC-LM3, Huh7, and HepG2 cells in a dose-dependent manner (P < 0.05 compared with the DMSO group) (Figure 1D and 1E).

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(Figure S1A-C) were consistent with those reported by a previous study [11]. PI3-K/Akt1 pathway is one of the pathways affected by DMF in systemic sclerosis fibrosis [22]. We found that DMF treatment decreased p-AKT and c-MYC levels in HCC cells (Figure 4A). To further investigate the mechanism of DMF in HCC cells, we analyzed the proteins that affected by DMF. DAVID bioinformatics database was used to perform Gene Ontology analysis to analyze the pathway regulated by DMF [23]. Gene Ontology analysis suggested that the JAK/STAT signaling pathway was associated with DMF treatment in HCC cells (Figure 4B).

Activation of STAT3 signaling increases tumor cell proliferation, survival, and invasion [12, 13]. Moreover, studies have also demonstrated the importance of the JAK/STAT pathway in HCC development. The JAK/STAT pathway is modulated by negative regulators such as SOCS proteins [24]. SOCS proteins can bind both cytokine receptors and JAK and are recruited to the tyrosine-phosphorylated receptor, facilitating inhibition of JAK [24, 25]. SOCS3, one of SOCS family members, is also likely involved in the suppression of tumor growth, vascular invasion, and metastasis of HCC [26, 27].

To demonstrate the effects of DMF on SOCS3/JAK1/STAT3 signaling pathway, HCC-LM3 and HepG2 cells were treated with 100 µM and 200 µM DMF for 48 h. Cells were harvested for western blot analysis with SOCS3/JAK1/STAT3 signaling pathway antibodies. Western blot analysis showed that in HCC-LM3 and HepG2 cells, after DMF treatment, SOCS3 protein expression was upregulated (Figure 4C and 4D), and phosphorylation of JAK1 (Figure 4C and 4D) and STAT3 (Figure 4A and 4B) was severely inhibited. Therefore, the results indicated that DMF activated SOCS3 protein expression and inhibited the JAK1/STAT3 signaling pathway with SOCS3 negative feedback, thus efficiently suppressing proliferation and angiogenesis in HCC. These results suggested that SOCS3/JAK1/STAT3 pathway was a potential mechanism of action in DMF-treated HCC cells.

Figure 3. DMF inhibited tube formation of HUVECs. A. DMF inhibited HUVECs tubulogenesis in vitro. B. The number of tubes treated with different concentrations of DMF. Values are presented as the mean ± SD, every experiment was repeated three times. *P < 0.05 and ***P < 0.001. C. Expression of angiogenesis related proteins p-VEGFR2 and VEGFA in HUVECs after treating with DMF were analyzed by western blot.
DMF suppresses HCC progression

Dimethyl fumarate decreases autophagy in HCC cells

STAT3 plays a crucial role in regulating autophagy. Inhibition of the STAT3 signaling pathway would decrease autophagy [18]. In the present study, we have demonstrated that DMF inhibited the STAT3 signaling pathway. Therefore, to explore the effect of DMF towards autophagy, we analyzed cells treated with different concentrations of DMF. The changes in autophagy markers Atg5, Atg7, and LC3B were all detected by western blot assays in HCC-LM3 and HepG2 cells. We found that Atg5, Atg7, and LC3B pro-

Figure 4. DMF activated the expression of SOCS3 and inhibited JAK1/STAT3 signaling. A. p-AKT and c-MYC were examined in HCC-LM3 and HepG2 cells after treating with DMF. B. Gene Ontology analysis of the signaling pathway regulated by DMF. Jak-STAT signaling pathway was found associated with DMF treatment in HCC cells. C and D. HCC-LM3 and HepG2 cells were treated with DMF for 48 hours, p-STAT3 and STAT3 were analyzed by western blot. E and F. HCC-LM3 and HepG2 cells were treated with DMF for 48 hours. SOCS3, p-Jak1 and Jak1 were evaluated by western blot.
DMF suppresses HCC progression

Protein levels significantly decreased (Figure 5A and 5B). In order to verify the inhibitory effects of DMF on autophagy, HepG2 cells were treated with autophagy inducer rapamycin or rapamycin combined with DMF. Autophagy was activated after treatment with rapamycin, whereas autophagy was inhibited after treatment with rapamycin combined with DMF (Figure 5C). To detect changes in autophagic flux induced by DMF, we transduced mCherry-GFP-LC3 adenovirus into Huh7 cells, and then treated the cells with rapamycin or rapamycin combined with DMF. Cells were observed under a fluorescence microscope. Autophagy was found to be activated in the rapamycin-treated group; in contrast, the level of autophagy decreased in the group treated with rapamycin combined with DMF (Figure 5D).

**Dimethyl fumarate suppresses tumorigenicity in vivo**

Anticancer property of DMF was further assessed in mice models. HCC-LM3 cells (3 × 10^6) were subcutaneously injected into 6-week-old male nude mice; tumor formation was monitored by palpation. When tumors could be detected, mice were randomized into either the control or the DMF treatment groups. The DMF treatment group showed significantly lower tumor size and body weight than the control group (Figure 6A-C).
DMF suppresses HCC progression

Figure 6. DMF suppressed the tumorigenicity of HCC cell lines in vivo. A and B. DMF inhibited the growth and weight of HCC tumors in nude mice. C. Photograph of xenograft tumors. D and E. Western blot assay were used to examine the expression of SOCS3, STAT3, p-STAT3, Jak1 and p-Jak1 in xenograft tumor tissue. F. Cell cycle-associated proteins CDK2, CDK4 as well as angiogenesis related proteins VEGF and p-VEGFR2 were detected by western blot in xenograft tumor tissue. G. Tumor sections for Ki67 and CD31 protein expression were analyzed by immunohistochemistry (magnification, × 200).
DMF suppresses HCC progression

Figure 7. DMF showed non-toxic effects on mice. A. Mice body weight of xenograft nude mice was measured. Values are presented as the mean ± SD, P > 0.05 was seen as no significant difference. B. Cecums were observed from macroscopic observation and H&E stain. C. Liver function related alkaline phosphatase (ALP), aspartate aminotransferase (AST) and aminotransferase (ALT) were examined. Values are presented as the mean ± SD. D. BUN, Cr and UA levels of kidney function index were measured. Values are presented as the mean ± SD. E. Erythrocytes, white blood cells and blood platelets levels of blood routine index were evaluated. Values are presented as the mean ± SD.
DMF suppresses HCC progression

To verify whether the mechanism of HCC inhibition by DMF in vivo is consistent with that observed in vitro, tumor tissues from the control and DMF treatment groups were harvested to carry out western blot analysis. We found that DMF activated SOCS3 protein and, sequentially abolished the phosphorylation of JAK1 and STAT3 (Figure 6D and 6E). With the suppression of JAK1/STAT3 signaling, cell cycle-associated proteins CDK2 and CDK4 were downregulated (Figure 6F). Moreover, the proteins involved in angiogenesis, VEGFA and p-VEGFR2, were also effectively inhibited (Figure 6F). These results demonstrated that molecular mechanisms of HCC inhibition by DMF in vivo and in vitro are consistent.

Immunohistochemistry showed that Ki67 expression in the DMF treatment group was lower than that in the control group (Figure 6G). This suggests that DMF effectively inhibited proliferation of HCC cells in vivo. Moreover, CD31 expression significantly decreased in the DMF group (Figure 6G), which indicates that DMF inhibited angiogenesis of HCC in vivo.

**Dimethyl fumarate shows good biocompatibility in vivo**

To verify the toxicity of DMF on mice, body weight was measured. No significant difference in body weight was observed between the DMF-treated group and the DMSO-treated group (Figure 7A).

Dimethyl fumarate, as an oral drug, was reported to have corrosive effects on human intestine. In order to examine whether DMF has corrosive effects on the intestine, we obtained mouse cecum from both the groups. In comparison with the control group, no significant corrosion was observed from macroscopic observations and H&E staining in the DMF-treated group (Figure 7B).

Liver function, renal function as well as routine blood counts of mice were examined with standard biochemical markers that indicate hepatic and renal cytolysis. Activity of ALP and AST in the DMF group was slightly lower than that in the control group (Figure 7C). However, ALT activity in the DMF group was higher than that in the control group (Figure 7C). Additionally, CR, BUN, and UA levels were monitored to examine renal function. However, no significant changes in renal function were found between the DMF group and the control group (Figure 7D). Routine blood counts in the DMF group also showed negligible changes (Figure 7E). These results suggest that treatment with DMF leads to low hepatic and renal cytolysis.

**Discussion**

In the present study, we demonstrated that SOCS3 was upregulated after DMF treatment, and then the phosphorylation of JAK1 and STAT3 decreased with SOCS3 overexpression. Consequently, inhibition of the JAK1/STAT3 pathway efficiently downregulated proliferation-associated factor Ki67 in xenograft tumor treating with DMF. Moreover, cell cycle-associated factors CDK2, CDK4, and cyclin D1 were downregulated. Protein levels of autophagy markers LC3B, ATG5, and ATG7 also decreased. Angiogenesis was also suppressed as suggested by the inhibition of VEGFA and p-VEGFR2 expression. The mechanism underlying induction of the SOCS3/JAK1/STAT3 signaling path-
DMF suppresses HCC progression

Hepatocellular carcinoma has a high mortality rate in developing countries, especially in China. Although many treatment options are currently available for HCC such as surgery, local chemotherapy, and liver transplantation—the treatment is still limited and the prognosis of HCC remains poor [28]. Therefore, it is necessary to find a novel treatment strategy for HCC. Dimethyl fumarate is a methyl ester of fumaric acid, a commonly used industrial compound that is inexpensive and easy to obtain. Studies have reported that DMF has anti-tumor effects on certain cancers [10, 11]. Our results suggest that DMF reduces the growth of HCC cells both in vivo and in vitro, and shows a relatively moderate toxicity for mice. Dimethyl fumarate also plays a role in inhibiting tumor migration, which may be due to tumor cell apoptosis induced by DMF [10].

The anti-tumor mechanism of DMF treatment is still rare. We explored a deeper mechanism for HCC treatment with DMF. SOCS3 expression plays a role in the negative feedback loop to attenuate the JAK1/STAT3 signaling pathway, inhibiting JAK1 activity and phosphorylation of STAT3 downstream [19]. Excessive activation of STAT3 is ubiquitous in many malignancies. However, STAT3 signaling also regulates tumor cell proliferation, migration, invasion, autophagy, and tumor angiogenesis [12, 13]. Therefore, inhibition of STAT3 is a viable therapeutic target for the treatment of HCC.

Autophagy is a process that maintains cellular homeostasis in response to a range of extracellular stress stimuli. STAT3 signaling pathway is involved in various aspects of the autophagy process. Different subcellular localizations of STAT3 have recently been reported to regulate autophagy in various manners [18]. Our data showed that DMF significantly inhibited the autophagy pathway as indicated by the decreased levels of the autophagic markers LC3B, Atg5, and Atg7. Moreover, DMF also inhibits autophagy caused by the autophagy inducer rapamycin. Dimethyl fumarate can also inhibit the formation of autophagic flux. In conclusion, these data indicated that DMF suppressed autophagy via inhibition of STAT3 signaling.

Tumor angiogenesis plays a vital role in HCC tumor invasion, metastasis, and progression [29]. VEGFA is an endothelial cell-specific growth factor and a key regulator of angiogenesis in tumor progression. These functions are primarily based on the interactions of VEGF with three structurally related tyrosine kinase receptors: VEGFR-1, VEGFR-2, and VEGFR-3. Extensive studies have been conducted to inhibit VEGF/VEGFR2 signaling to prevent angiogenesis [30]. Markus’s study provides evidence for suppression of VEGFR2 expression in human endothelial cells by DMF treatment [31]. This finding was also supported by our data. Accordingly, controlling tumor angiogenesis remains a crucial target for the successful treatment of HCC.

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Disclosure of conflict of interest

None.

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References

DMF suppresses HCC progression


DMF suppresses HCC progression

Figure S1. DMF promotes the apoptosis of HCC cells. A. The apoptosis level of Huh7 cells were increased with DMF treatment. B. Percentage of apoptotic HCC cells, Data is expressed as mean ± SD, **P < 0.01, ***P < 0.001. C. DMF decreased the expression of Bcl-xl in HCC-LM3 and HepG2 cells with treatment of DMF.