Dimethylfumarate Impairs Melanoma Growth and Metastasis

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Abstract

Dimethylfumarate (DMF) inhibits signals transmitted by Rel proteins and is used for the treatment of inflammatory skin diseases such as psoriasis, but potential effects of DMF on tumor progression have yet not been analyzed. We show that DMF reduced melanoma growth and metastasis in severe combined immunodeficient mouse models. To identify targets of DMF action, we analyzed mRNA expression in DMF-treated melanomas by gene chip arrays. Using BiblioSphere software for data analysis, significantly regulated genes were mapped to Gene Ontology terms cell death, cell growth, and cell cycle. Indeed, we found that DMF inhibited proliferation of human melanoma cell lines A375 and M24met in vitro. The cell cycle was arrested at the G2-M boundary. Moreover, DMF was proapoptotic, as shown by cell cycle analysis and by Annexin V and Apo2.7 staining. These results were confirmed in vivo. DMF reduced proliferation rates of tumor cells as assessed by Ki-67 immunostaining and increased apoptosis as assessed by terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling staining. In conclusion, DMF is antiproliferative and proapoptotic and reduces melanoma growth and metastasis in animal models. (Cancer Res 2006; 66(24): 11888-96)

Introduction

The Rel protein family of transcription factors consists of five members, which all share a so-called rel-homology domain. In human, members of this protein family are p50, p52, p65/RelA, RelB, and c-rel. In normal cells, these proteins are located within the cytosol in a complex with inhibitory proteins (IκB-proteins, p100, p105, or bel-3). On activation, dimers of Rel proteins (predominantly p65/p50 or p65/c-Rel) are released; they translocate into the nucleus and induce gene transcription. Following shutdown of the activation signal, the nucleus is cleared from Rel protein dimers and cells return into a quiescent state. The situation is different in cultured tumor cells, where a constitutive activation of this signaling pathway can be found (1–5). In such cells, expression of downstream targets is constitutively induced (cytokines, metalloproteinases, cell cycle proteins, and apoptosis-regulating genes), all of which directly or indirectly promote tumor progression (6, 7). Conversely, suppression of transcriptionally active Rel proteins has been shown to inhibit proliferation, cause cell cycle arrest, and induce apoptosis (8–11).

Animal models have provided a direct link between activation of Rel proteins and tumor progression. For example, melanoma cells expressing high levels of constitutive active p65/RelA are highly metastatic in a mouse model. Transfection of tumor cells with dominant-negative mutants of p65/RelA reduced melanoma growth and lung metastasis (12), reduced tumor burden in a Lewis lung cancer model (13), and enhanced radiosensibility of melanoma cells (2). In addition, compounds that inhibit Rel signaling have been used in tumor models. Dehydroxyethylphosphorylquinomycin inhibits tumor progression in murine models for T-cell leukemia and multiple myeloma (14, 15) and curcin inhibits metastasis of breast cancer in a nude mouse model (16).

This has led to the concept that constitutive activation of the Rel signaling pathway is involved in cancer progression as reviewed by several authors (7, 17–19). Currently, a direct relationship between activity of this signaling pathway and tumor progression in humans is difficult to establish. Nevertheless, different phase I/II clinical trials have been initiated recently. The proteasome inhibitors bortezomib and PS-341 and the inhibitor of IκB phosphorylation, Xenavex, are now being tested in human patients with cancer.

Dimethylfumarate (DMF) inhibits p65/RelA activity by blocking nuclear translocation of activated Rel protein dimers (20–24). We here analyze effects of DMF on melanoma growth and metastasis. The rationale for choosing this malignancy was that (i) in melanoma cell lines as well as in melanomas in vivo, this signaling pathway is constitutive active (5, 25); (ii) metastatic melanoma has poor prognosis; and (iii) currently, no treatment is able to improve 5-year survival (26). Using humanized severe combined immunodeficient (SCID) mouse melanoma models, we show that DMF reduced growth and metastasis based on its antiproliferative and proapoptotic actions.

Materials and Methods

Cells, Antibodies, and Reagents

Human melanoma cell lines A375 (ATCC; LGC Promochem GmbH, Wesel, Germany) and M24met (kindly provided by Dr. R.A. Reifsfeld, Department of Immunology, Scripps Research Institute, La Jolla, CA; ref. 27) were cultivated in Iscove’s modified Dulbecco’s medium (IMDM) supplemented with 10% FCS, 2 mmol/L L-glutamine, and 50 IU/mL penicillin-streptomycin (all from Life Technologies, Vienna, Austria).

Monoclonal mouse anti-human Ki-67 (clone Ki67) and mouse anti-human Apo2.7 (clone 2.7A6A3) antibodies were from Beckman Coulter (Fullerton, CA). Monoclonal mouse anti-human vimentin antibody (clone V9), isotype control antibodies, and biotinylated second step antibodies were purchased from Dako Denmark A/S (Glostrup, Denmark). Detection of biotinylated secondary antibodies was done with peroxidase/AEC REAL Detection System from Dako. R-phycocerythrin-labeled mouse anti-human interleukin-8 (IL-8) monoclonal antibody was from BD PharMingen (Vienna, Austria).

R-phycocerythrin-conjugated Annexin V and SYTOX Green nucleic acid stain were from Invitrogen, Molecular Probes (Eugene, OR).

DMF (Fumapharm, Muri, CH) was solubilized in methanol as a 70 mmol/L stock solution and diluted in IMDM for final concentrations. The solvent (methanol) was routinely used as a control in all experiments. All stock solutions were stored at 4°C and used within 24 hours.

Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

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1 http://www.clinicaltrials.gov.
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**SCID Mouse Models**

All procedures were carried out in accordance with the Association for Assessment and Accreditation of Laboratory Animal Care guidelines and Guide for the Care and Use of Laboratory Animals (Department of Health and Human Services, NIH, Publication no. 86-23). In addition, all experiments were approved by the ethics committee of the Medical University of Vienna and by the Austrian government committee on animal experimentation.

Pathogen-free, 4- to 6-week-old female CB17 scid/scid (SCID) mice (Charles River, Sulzfeld, Germany) were housed and used as described (28). For the nodular tumor model, A375 melanoma cells suspended in PBS were injected s.c. into the right flank of the animals (numbers of injected cells are indicated in each experiment). After clinical appearance of nodular tumors, DMF at indicated concentrations suspended in 0.8% methylcellulose was orally administered daily via gauge. Controls received 0.8% methylcellulose alone. Tumor volume was assessed daily as previously described (28). At indicated times, animals were sacrificed and tumors including the surrounding skin were explanted. Specimens were divided into two pieces, one was fixed in 4% paraformaldehyde and embedded in paraffin and the other was transferred into RNAlater (Ambion, Austin, TX) and stored at −70°C.

For the metastatic tumor model, M24met cells were injected into the right flank of the animals. Treatment was initiated after clinical appearance of tumors as described above. When the tumors had reached a volume of 500 to 900 mm³, animals were anesthetized, tumors removed, and skin defects were sutured. Ten days later (day 22 after onset of treatment), animals were sacrificed. Lymph nodes and lungs were screened for macrometastasis by macrosectioning of organs. Tissues were fixed with 4% paraformaldehyde and embedded in paraffin. Preceding experiments (data not shown) were the basis for this protocol; in untreated animals, pulmonary metastasis was virtually absent 10 days after excision of primary tumors and ~ 50% of axillary and inguinal lymph nodes were populated with M24met cells. Waiting for longer period of times results in exuberant pulmonary tumor seeding, which is difficult to quantify. Other organs like brain, kidney, liver, or para-aortal/pelvic lymph nodes were never populated by M24met cells within the time frame of the experiments.

**cDNA Arrays**

Briefly, RNA was prepared from nodular tumors of A375 cells grown in SCID mice. Total RNA from five animals treated with DMF (20 mg/kg/d), five animals treated with DMF (6 mg/kg/d), and five controls treated with methylcellulose was used for microarray analysis. Fifteen gene chip arrays containing 45,000 probe sets of 33,000 genes (human HG-U133A, 50% of axillary and inguinal lymph nodes were populated with M24met cells. Waiting for longer period of times results in exuberant pulmonary tumor seeding, which is difficult to quantify. Other organs like brain, kidney, liver, or para-aortal/pelvic lymph nodes were never populated by M24met cells within the time frame of the experiments.

**Histology and Immunohistochemistry of Melanoma and Lymph Nodes**

Paraffin-embedded primary melanomas and lymph nodes were stained with H&E according to standard procedures.

**Detection of micrometastasis in lymph nodes.** Paraffin-embedded tissues were serially sectioned, dried at 80°C for 60 minutes, and deparaffinized according to routine procedures. Sections were then incubated in citrate buffer (pH 6.0) and microwaved for 15 minutes. Staining was done in a DAKO TechMate Horizon automated staining system according to the manufacturer’s protocol. Briefly, serial sections at steps of 30 μm (at least five sections per lymph node) were incubated with an anti-human vimentin antibody made in rabbit (which reacts with all human melanoma cells but does not recognize mouse vimentin) followed by incubation with a biotinylated antirabbit antibody. Bound antibodies were visualized by incubation with a streptavidin-horseradish peroxidase (HRP) conjugate (Dako) and 3- amino-9-ethylcarbazole (Dako), resulting in a red reaction product. Specimens were counterstained with Meyer’s hemalaun. The anti-vimentin antibody was chosen because anti-melanocytic antibodies like anti-S100, anti-HMB45, anti-Melan A, or anti-tyrosinase proved insensitive or even negative for lymph node metastasis of M24met cells (data not shown).

**Cell Cycle Analysis**

Cell cycle analysis was done as described elsewhere (29). Briefly, A375 and M24met human melanoma cells were grown in culture medium in the presence or absence of DMF for 4 to 72 hours. Then, cells were washed with ice-cold PBS, detached with trypsin/EDTA, washed, and resuspended in 200 μL of PBS and fixed in 70% ethanol for 30 minutes at 4°C. After treatment with 0.5 mg/mL RNase A for 20 minutes at 37°C, DNA was stained with propidium iodide dissolved in 0.1% sodium citrate (pH 8.0) for 10 minutes at 4°C. After passing the cells through a 0.4-μm nylon mesh to remove clots, cells were analyzed on a Spectra Max GeminiXS (Molecular Devices Corp., Sunnyvale, CA) with excitation/emission at 480/520 nm and data were analyzed using SoftMax PRO 4.0 software (Molecular Devices).
at room temperature in the dark according to the manufacturer’s protocol. A FACScan flow cytometer was used for data acquisition (Becton Dickinson), Cell Quest software was used for data analysis (Becton Dickinson).

Rel Protein–Dependent Reporter Gene Expression
A375 human melanoma cells were grown to 80% confluency. For transfection, 2 μg Rel protein responsive reporter construct (described in ref. 21), 0.2 μg TK-Renilla (according to the manufacturer’s protocol), 4 μL Lipofectamine 2000 reagent (Invitrogen), and 500 μL OptiMEM I (Invitrogen) were added per well. Two days after transfection, cells were stimulated with tumor necrosis factor (TNF)-α (25 ng/ml; Strathman Biotech, Hamburg, Germany) for 2 hours with or without preincubation with 84 μmol/L DMF for 2 hours. Cells were then lysed in 150 μL of passive lysis buffer using the Dual-Luciferase Reporter Assay System (Promega, Vienna, Austria) according to the manufacturer’s instructions. The Luciferase activity was measured with a Berthold Centro LB 960 luminometer and monitored with MikroWin 2000 software. Data were reported as normalized averages of the Luciferase/Renilla ratio.

IL-8 Protein and mRNA Expression In vitro and In vivo
For in vitro experiments, human A375 melanoma cells were preincubated with or without 84 μmol/L DMF for 2 hours and stimulated with TNF (25 ng/mL) or left untreated. Additionally, IL-8 secretion was inhibited by the addition of 1 μL/mL Golgi plug and Golgi stop (BD Biosciences, Vienna, Austria). FACS analysis was done after 6 hours; IL-8 was detected with an R-phycocerythrin-labeled monoclonal antibody.

For in vivo experiments, RNA obtained from tumor specimens from the experiment shown in Fig. 1 was extracted with the RNeasy Mini Kit (Qiagen, Vienna, Austria). RNA was reverse transcribed into first strand cDNA using Revert Aid M-MuLV Reverse Transcriptase (Fermentas, Vienna, Austria) and hexamer primers (Roche) for 90 minutes at 42°C. TaqMan real-time PCR was done in doublets. The primer sets used were from Applied Biosystems (Assays-on-Demand). A 25-μL reaction for each primer set was assembled with 2 μL of the reverse transcriptase reaction and TaqMan Universal PCR Master Mix (Applied Biosystems). A glyceraldehyde-3-phosphate dehydrogenase primer set was used to normalize the results for each sample tested. Reactions were run on ABI Prism 7700 Sequence Detector (Perkin-Elmer, Applied Biosystems).
Vienna, Austria) and data analysis was done with the SDS 1.9.1 software package (Applied Biosystems).

Statistical Analysis

The statistical comparison between individual data points was done by two-sided Student's t test. For the overall growth of melanoma in SCID mice, comparison was done by variance analysis (ANOVA for repeated measurements). Differences in lymph node metastases were calculated by using the two-tailed Fisher's exact test. \( P < 0.05 \) was considered statistically significant. All calculations were done with SPSS 14 software (SPSS, Inc., Chicago, IL).

For the determination of significantly regulated genes, ChipInspector analysis was carried out to circumvent annotation errors and errors due to the existence of alternative transcripts (refs. 30, 31; see Supplementary Fig. S1).

Results

DMF reduces melanoma growth and metastasis in SCID mouse models. First, DMF was tested in a nodular melanoma model using human A375 melanoma cells. Following s.c. injection of \( 1 \times 10^6 \) (Fig. 1) or \( 2 \times 10^5 \) (data not shown) cells into the right flank of CB17 SCID mice, palpable tumors developed after 1 or 2 weeks. At this time, treatment with DMF was initiated. Two different concentrations of DMF were used. In the high-dose group (20 mg/kg/d), the oral concentration of DMF in our mice was thrice that of the human dose for psoriasis patients (as measured per kilogram body weight). The low-dose group (6 mg/kg/d) corresponds to the human dose given orally to psoriatic patients (as measured per kilogram body weight). At a DMF dose of 20 mg/kg/d, tumor growth was significantly inhibited as compared with controls (\( P < 0.05 \)), irrespective of the numbers of A375 cells initially injected s.c. (Fig. 1). At a dose of 6 mg/kg/d, DMF insignificantly reduced tumor growth (data not shown).

Second, DMF was tested in a metastatic melanoma model using human M24met melanoma cells. M24met cells were derived from a lymph node metastasis of human melanoma (27). Following s.c. injection of \( 1 \times 10^6 \) cells into the right flank of mice, palpable tumors developed within 1 week (data not shown). At this time, treatment with DMF (20 mg/kg/d) was initiated. After 12 days of therapy, primary tumors were excised. Mean tumor volumes in the DMF group were \( 394 \pm 60 \text{ mm}^3 \) as compared with \( 569 \pm 107 \text{ mm}^3 \).

**Figure 3.** DMF reduces cell growth in vitro and alters cell cycle distribution. A375 and M24met human melanoma cells were treated with indicated concentrations of DMF or solvent control. **A,** assessment of cell proliferation by using a CASY1 TT cell counter and analyzer system. Columns, mean of three independent experiments, each done in triplicates; bars, SD. **B,** assessment of cell proliferation by measuring DNA content (CyQUANT Cell Proliferation Assay). Points, mean of three independent experiments, each done in triplicates; bars, SD. **C and D,** analysis of cell cycle as assessed by FACS using propidium iodide-stained human A375 and M24met cells; cells were treated for indicated times with 84 \( \mu \text{mol/L} \) DMF dissolved in methanol. **C,** representative example of FACS curves. **D,** summary of five experiments, each done in duplicates.
in controls \( (P = 0.14; \text{data not shown}) \). Treatment was continued for another 10 days and then animals were sacrificed. In 12 DMF-treated animals, 77\% of all axillary and inguinal lymph-nodes could be excised \( (n = 36; 12 \text{ were not found}) \). Eight animals were without lymph node metastasis, four had 1, and none had more than one positive lymph node. In the six controls, 75\% of all lymph nodes could be excised \( (n = 18; 6 \text{ were not found}) \). Two animals were without lymph node metastasis, two had 1, one had 2, and one had 3 positive lymph nodes \( (\text{Fig. 2A and B}) \). Using the two-tailed Fisher’s exact test (lymph nodes not found were not included in this calculation) revealed a statistically significant difference \( (P = 0.029) \).

Lungs were also serially sectioned. In none of the lungs melanoma cell clusters were detectable by H&E staining. By immunohistochemistry with anti-vimentin antibodies, single vimentin-positive cells (no tumor cell clusters) were found in 1 of 6 controls and in 1 of 12 DMF-treated animals (data not shown).

DMF alters gene expression profiles in melanoma. Total numbers of significantly regulated genes in 6 mg/kg/d DMF-treated animals were 209 and in 20 mg/kg/d DMF-treated animals, 527. To evaluate the effects of DMF on gene expression, data sets from 2 \( \times 5 \text{ DMF-treated (6 mg/kg/d; 20 mg/kg/d) and 5 control gene chips were first analyzed for their } \)sample distance” (measure of similarity in the gene expression profile). The hypothetical tree-like diagram shown in Supplementary Fig. S1 scales distances between data sets; a short distance between samples describes a close relationship. DMF-treated animals and controls largely fall into three virtual clusters (control, low-dose, and high-dose treatment), confirming that DMF alters gene expression and this effect is dose related. Data sets were then analyzed by ChipInspector and BiblioSphere PE software and mapped to Gene Ontology trees. The highest numbers of regulated genes (irrespective of up- or down-regulation) were obtained in categories cell death, cell
growth, and cell cycle (Supplementary Fig. S1; Supplementary Files 1 and 2). These results guided us to investigate the effects of DMF on proliferation and apoptosis in melanoma cells in vitro.

**DMF effects on proliferation, cell cycle, and apoptosis in vitro.** DMF reduced proliferation of A375 and M24met melanoma cells in a dose-dependent fashion. This was analyzed by two independent assays, one counting the number of cells and the other quantifying the amount of DNA. In both assays, concentrations of 84 μmol/L DMF completely inhibited cell growth (Fig. 3A and B). By contrast, control-treated A375 cells increased their cell numbers within 6 days by a factor of 28 (±9) and M24met cells by a factor of 9 (±1).

DMF altered the cell cycle in A375 and M24met cells. After 48 hours, ~60% of A375 cells and 25% of M24met cells accumulated within the G2-M peak (Fig. 3C and D). In controls, 90% of A375 and M24met cells were within G0-G1 and few cells were within S or G2-M, which is in line with previously published findings (32, 33). In addition, the amount of cells within the so-called sub-G1 phase, a hallmark for apoptosis, increased in DMF-treated cells (Fig. 3C and D).

**DMF effects on proliferation and apoptosis in vivo.** Ki-67 antigen expression was analyzed on tissue sections derived from nodular melanomas grown in SCID mice. DMF significantly reduced the numbers of Ki-67-positive A375 cells (35.6, SD ±15.0, Figure 5. DMF is antiproliferative and proapoptotic in melanoma in vivo. A, immunochemistry of primary A375 tumors treated with 20 mg/kg/d DMF or methylcellulose orally as described in Materials and Methods. Paraformaldehyde-fixed, paraffin-embedded 5-μm sections were stained with mouse anti-human Ki-67 antibody (red) and counterstained with hemalaun (blue). In each section, five high-power fields (HPF) were randomly photographed and numbers of Ki-67-positive cells were counted by two independent observers blinded to the conditions. In the diagram at the right side, each dot represents one high-power field. The difference between groups was significant, P < 0.001. Points, mean; bars, SE. B, TUNEL staining of A375 primary tumors treated with 20 mg/kg/d DMF or methylcellulose orally as described in Materials and Methods. Paraformaldehyde-fixed, paraffin-embedded 5-μm sections were used for the assay. TUNEL-positive nuclei are in green; all nuclei stained with propidium iodide are in red. In each section, two high-power fields were randomly photographed and percent of TUNEL-positive cells out of the total number of propidium iodide positive cells was evaluated by two independent observers blinded to the conditions. The summary is shown in the diagram on the right. The difference between groups was significant, P < 0.001. Points, mean; bars, SE.
SE ± 1.92 per high-power field) as compared with controls (45.8, SD ± 15.7, SE ± 1.59; P < 0.001; Fig. 5A and B). Also in primary tumors of M24met cells, numbers of Ki-67-positive cells were significantly reduced in DMF-treated animals (18.9, SD ± 10.1, SE ± 1.7) as compared with controls (27.2, SD ± 14.0, SE ± 1.1; P < 0.001; Fig. 5B). It has to be noted that Ki-67 is detectable throughout the cell cycle, including those cells in the G2-M. However, in vivo, we have shown that DMF leads to an accumulation of cells in G2-M (Fig. 3D). It is thus conceivable that true proliferation rates in DMF-treated melanoma were even lower than those determined by Ki-67 staining.

Terminal deoxynucleotidyl transferase–mediated dUTP nick end labeling (TUNEL) staining was done on tissue sections derived from nodular melanomas grown in SCID mice. In DMF-treated animals, 4.3 ± 0.7% of tumor cells were TUNEL positive, whereas in controls only 0.8 ± 0.2% of tumor cells were TUNEL positive (P < 0.001; Fig. 5C). In primary M24met tumors (excised at day 12 of treatment), numbers of TUNEL-positive cells did not differ between treatment groups (data not shown).

**DMF inhibits Rel protein–dependent functions in vitro and in vivo.** Basal expression of a Rel protein reporter construct transfected into A375 cells was significantly reduced by 84 μmol/L DMF as compared with cells treated with solvent control. On stimulation with TNF, expression was induced in control cells and this was also significantly inhibited by DMF (n = 4, each done in triplicate; Fig. 6A). We next analyzed TNF-induced IL-8 expression (a protein predominantly regulated by rel proteins; refs. 34, 35). IL-8 protein expression in A375 cells was evaluated by FACS (representative example shown in Fig. 6B). The TNF-induced increase in IL-8 expression was significantly inhibited by DMF (n = 4; Fig. 6B). We next analyzed IL-8 mRNA expression in melanoma in vivo 2 and 10 days after grafting onto SCID mice. We used human primers that did not amplify mouse IL-8 mRNA. We found a significant reduction of IL-8 mRNA in DMF-treated animals as compared with controls at both time points (Fig. 6C).

**Discussion**

Standard procedures in the therapy of melanoma aim to destruct tumor cells by chemotherapy, radiotherapy, or surgical removal. Even combinations thereof failed to improve survival of patients with metastatic melanoma (36). For this reason, alternative approaches for the treatment of melanoma are needed. Here we show that DMF significantly delayed melanoma growth in a SCID mouse model. Moreover, in a second model, DMF significantly delayed, but did not prevent, lymphogenic tumor spread. We have chosen the xenograft model because it allowed identification of genes regulated by DMF in melanoma cells in vivo. By using human cDNA arrays, we have identified genes regulated in human melanoma cells and largely excluded genes expressed in the mouse stroma, thereby reducing the heterodyning effects of mouse genes.

By Gene Ontology analysis, most numbers of significantly up-regulated or down-regulated genes were found within terms cell death, cell growth, and cell cycle. This held true for both the low-dose and high-dose DMF groups. By analyzing gene expression of individual genes, the magnitude of difference between mRNA expression levels in DMF-treated animals and controls did not exceed a 4-fold change (data not shown). This indicates that under
the condition of continuous treatment, DMF exerts its function by tuning expression profiles of interleaved genes and not by pronounced up-regulation or down-regulation of individual targets. To analyze the biological consequences of this DMF-induced tuning of genes controlling growth and death, we characterized the effects of DMF on melanoma cells in vitro. We show that DMF inhibited proliferation, induced a cell cycle arrest at the G2-M boundary, and induced apoptosis in cultured melanoma cells. Results from in vitro studies were confirmed in vivo. By doing immunohistochemistry, DMF decreased the numbers of Ki-67-positive melanoma cells and increased the numbers of TUNEL-positive cells, indicating that DMF is also antiproliferative and proapoptotic in vivo.

This raises the question of how DMF interferes with tumor growth and apoptosis. We and others have shown that DMF interferes with Rel protein–dependent gene transcription in normal cells (20–24). Here we show that DMF inhibits Rel protein–dependent reporter gene expression, as well as TNF-induced IL-8 expression, in melanoma cells in vitro and in vivo, confirming that DMF is also active in transformed cells. There is an interesting analogy between DMF and curcumin. Both inhibit Rel signaling, arrest cell cycles in G2-M, and induce apoptosis in human melanoma cells (11, 25). Taken together, these data are in line with the emerging concept that inhibition of Rel proteins reduces cell proliferation and induces apoptosis in cancer (7, 37). However, with regard to the mechanism of action of DMF, the situation is complicated by a recent evidence that DMF also interferes with the intracellular redox system [recently reviewed by Mrowietz et al. (38)]. For oxidants, cyclin D1 is a primary regulatory node for the induction of cell growth (39). We have searched our cDNA array database and did not find reduced cyclin D1 mRNA expression in DMF-treated animals as compared with controls, which favors the assumption that DMF delays tumor progression by inhibition of the Rel protein–dependent signaling pathway.

In conclusion, we show that DMF is antiproliferative and proapoptotic in melanoma cells and delays melanoma progression and metastasis in animal models. The effective dose of DMF in our animal models is only thrice that of the dose used in psoriatic patients. Due to collected safety data of fumarates in long-term treatment of psoriasis (40, 41) and the oral delivery route, DMF is an attractive candidate for a phase II clinical trial in human patients with stage III melanoma. Because DMF delays, but does not prevent, tumor spread, this compound will have to be combined with other therapies. Candidates are alkylating agents because excessive activation of Rel proteins has been established as a principal mechanism of tumor chemoresistance (42–44).

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References


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Dimethylfumarate Impairs Melanoma Growth and Metastasis

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