Curcumin Induces the Differentiation of Myeloid-Derived Suppressor Cells and Inhibits Their Interaction with Cancer Cells and Related Tumor Growth

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Abstract
Myeloid-derived suppressor cells (MDSC) accumulate in the spleen and tumors and contribute to tumor growth, angiogenesis, and progression. In this study, we examined the effects of curcumin on the activation and differentiation of MDSCs, their interaction with human cancer cells, and related tumor growth. Treatment with curcumin in the diet or by intraperitoneal injection significantly inhibited tumorigenicity and tumor growth, decreased the percentages of MDSCs in the spleen, blood, and tumor tissues, reduced interleukin (IL)-6 levels in the serum and tumor tissues in a human gastric cancer xenograft model and a mouse colon cancer allograft model. Curcumin treatment significantly inhibited cell proliferation and colony formation of cancer cells and decreased the secretion of murine IL-6 by MDSCs in a coculture system. Curcumin treatment inhibited the expansion of MDSCs, the activation of Stat3 and NF-kB in MDSCs, and the secretion of IL-6 by MDSCs, when MDSCs were cultured in the presence of IL-1β, or with cancer cell- or myofibroblast-conditioned medium. Furthermore, curcumin treatment polarized MDSCs toward a M1-like phenotype with an increased expression of CCR7 and decreased expression of dectin 1 in vivo and in vitro. Our results show that curcumin inhibits the accumulation of MDSCs and their interaction with cancer cells and induces the differentiation of MDSCs. The induction of MDSC differentiation and inhibition of the interaction of MDSCs with cancer cells are potential strategies for cancer prevention and therapy. Cancer Prev Res; 5(2); 205–15. ©2011 AACR.

Introduction
Myeloid-derived suppressor cells (MDSC) have been identified as a heterogeneous population of immature myeloid cells with the ability to regulate innate immune responses and suppress T-cell activation in humans and animal models (1–4). MDSCs, accumulated in the blood, lymph tissues, and tumor sites have been shown to promote tumor growth, angiogenesis, and metastasis (5–8). Our previous results show that the mobilization and activation of MDSCs are critical early events for interleukin (IL)-1β–induced gastric carcinogenesis (6). IL-1β directly activates MDSCs through the NF-κB pathway, resulting in enhanced IL-6 production in MDSCs. Inhibition of MDSC activation by NF-κB inhibitor reduced the production of IL-6, inhibited MDSC mobilization, and suppressed the development of gastric cancer in IL-1β transgenic mice (7). These data suggest that inhibition of MDSC activation may be an effective approach for gastric cancer prevention.

The expansion and activation of MDSCs are influenced by a number of factors (9, 10). Most of these factors trigger signaling pathways in MDSCs that converge on Janus kinase (JAK) protein family members and Stat3 (11, 12). MDSCs from tumor-bearing mice have markedly increased levels of phosphorylated Stat3 (p-Stat3) compared with immature myeloid cells from naive mice (12). Moreover, ablation of Stat3 expression markedly reduces the expansion of MDSCs and increases T-cell responses in tumor-bearing mice (12, 13). These data elucidate the important role of Stat3 in MDSC function. Some approaches have been explored to inhibit MDSC activation, promote MDSC differentiation, and decrease MDSC expansion and accumulation (1, 10, 14–18). For example, selective depletion of Gr−1− MDSCs by in vivo administration of monoclonal antibodies against Gr−1− has resulted in restoring T-cell antitumor activity (19). The potential risk of this approach is that...
tumor-bearing hosts treated with such depleting antibodies may undergo opportunistic infections because of granulocyte deficiency. Thus, searching for safe and effective agents to inhibit MDSCs for cancer prevention is of importance.

Curcumin, derived from the perennial herb *Curcuma longa Linn*, exhibits cancer preventive and therapeutic properties (20–23). It has a long history of human use in food and as a medication. Curcumin has been shown to be an antitumor agent by modulating multiple targets (20), including suppressing the activity of NF-κB (24–27) and JAK2/Stat3 signaling in tumor cells and immune cells (26, 28). Curcumin is also a potent immunomodulator (24). However, the effect of curcumin on MDSCs remains to be studied.

Our previous study has shown that IL-1β induces MDSC activation to produce IL-6 through the NF-κB pathway (6). The IL-6 secreted by MDSCs may directly activate a Stat3 pathway in cancer cells and promote tumor growth (18). However, the interactions between MDSCs and cancer cells have not been adequately delineated. In this study, we investigated such interactions and their inhibition by curcumin. Our results show that curcumin inhibits the accumulation and activation of MDSCs, interferes with the interaction between gastric cancer cells and MDSCs, induces differentiation of MDSCs, and suppresses tumor growth.

Materials and Methods

**Cell culture and reagents**

Human gastric cancer cell line MKN-45 purchased from RIKEN (29, 30) was verified by morphology, growth curve analysis, and tested for *Mycoplasma* in March 2010. Mouse colon cancer cell line CT26 was obtained from American Type Culture Collection in June 2010. We did not do further characterization. Bone marrow–derived gastric myofibroblasts (BMD-MF) from IL-1β transgenic mouse were generated in our laboratory (31, 32). EGFP⁺ MKN-45 cells were generated through infection of EGFP-retrovirus in our laboratory. *Mycoplasma* negative in all 4 cell lines was confirmed by a PCR method (Stratagene) in April 2011. All cell lines were maintained in a RPMI-1640 medium containing 10% FBS, 100 U/mL penicillin, and 100 μg/mL streptomycin (Gibco BRL). Curcumin (a gift from Sabinsa Corporation) was dissolved in dimethyl sulfoxide (DMSO) and stored at −20°C.

**Tumor graft models and treatments**

Four-week-old athymic nude mice and BALB/c mice from Jackson Laboratory were maintained on the AIN93M diet in a sterilized animal room in our animal facility. Gastric cancer cells MKN-45 (1 × 10⁶) were injected subcutaneously into the flank of athymic nude mice. Mouse CT26-colican cancer cells (1 × 10⁵) syngenic to BALB/c mice were injected subcutaneously into the flank of BALB/c mice. Starting on the same day of injection, the athymic nude or BALB/c mice were put on the 2% curcumin diet (20 g curcumin/kg AIN93M diet) for 4 weeks. In another study, the mice received daily intraperitoneal injection of curcumin at 50 mg/kg body weight for 3 weeks after tumors reached a size of 100 mm³. Tumor volume was measured by a caliper once every 3 days. Spleens, blood, and tumor tissues were harvested at the end of the experiments. Animal protocols were approved by the Animal Care and Facilities Committee (ACFC) of Rutgers, The State University of New Jersey (Protocol No. 09–050).

**Detection of MDSCs in the spleens, blood, and tumor tissues**

The isolation of MDSCs from spleens, blood, and tumor tissues was done as described previously (7). In brief, splenocytes were obtained by passing through a 40-μm cellular strainer (BD Biosciences). Total nucleated cells in peripheral blood were isolated after erythrocyte lysis. To obtain tumor-infiltrating cells, tumors were cut into small pieces and digested with 5 mg/mL collagenase type IV (Sigma-Aldrich) at 37°C for 15 minutes. Cells were passed through a 40-μm cellular strainer. Cell suspensions were stained with fluorescence-labeled antibodies, then subjected to fluorescence-activated cell sorting (FACS) analysis using FACS 300 or MoFlo cell sorter (Beckman Coulter) at the Cytometry and Imaging Core Facility. All antibodies for FACS analysis were purchased from eBioscience. Data were analyzed using the Summit software (Beckman Coulter).

**Studies with MDSCs and cancer cells In Vitro**

Splenic MDSCs from mice were sorted by MoFlo cell sorter (Beckman Coulter) using PE-CD11b and PE-cy7-Gr-1 antibodies; or by MACS Separators using magnetic labeled MDSC isolation kit (Miltenyi Biotec). EGFP⁺ MKN-45 cells (1 × 10⁵ per well) was cocultured with splenic MDSCs (1 × 10⁶ per well) in a RPMI 1640 complete medium in the absence or presence of different concentrations of curcumin. Forty-eight hours after coculture, cell colonies were counted under a light microscope or fluorescence microscope. Cell supernatant was collected for ELISA analysis. In some experiments, MDSCs were cultured in the presence or absence of IL-1β for 36 hours, or with normal medium (NM) or cancer cell conditional medium (CC-M), or myofibroblast-medium (MF-M) in the presence or absence of curcumin for 48 hours. Culture medium was collected for ELISA. Cells were harvested for FACS analysis, or for extracting proteins and mRNA.

**Measurement of cytokine levels by ELISA**

The levels of murine IL-6 in the supernatant, serum and xenograft tumor tissues were determined by using an ELISA Kit (BD Biosciences). Absorbance was measured at 450 nm by a Multiscan MC reader, and the samples were analyzed by DELTA SOFT II software (BioMetallics).

**Real-time PCR**

RNA was extracted with TRIzol (Invitrogen). cDNA was synthesized (6). Real-time PCR was carried out with 1 μL cDNA, TaqMan Universal PCR Master Mix (Applied
Biosystems), and target gene assay mix containing sequence-specific primers and 6-carboxyfluorescein dye-labeled TaqMan minor groove binder probe (Applied Biosystems). Data quantitation was done using the relative standard curve method. Expression levels of the genes were normalized to glyceraldehyde 3-phosphate dehydrogenase.

**Apoptosis assays**

To detect in vivo apoptosis of MDSCs, splenocytes were isolated from tumor-bearing mice. Splenocytes were stained with APC-CD11b, PE-cy7-Gr-1, PE-Annexin V, and 7-AAD and then cells were analyzed by FACS. The apoptotic cells were counted among the CD11b⁺Gr-1⁺ population.

**Western blot analysis**

MDSCs were lysed with lysis buffer. Protein samples were subjected to SDS-polyacrylamide gels (Bio-Rad) electrophoresis. The gels were transferred onto nitrocellulose membranes (Bio-Rad). The membranes were probed with specific primary antibodies against Stat3, p65, p-Stat3, p-p65, iNOS, survivin, COX-2, and β-actin (Cell Signaling Technology), incubated with secondary antibodies conjugated to IR fluorophore, Alexa Fluor 680 (Molecular Probes), or IRdye 800 (Rockland Immunochemicals), and scanned using the Odyssey Infrared Imaging System (Li-Cor Biosciences).

**Statistical analysis**

Unless indicated otherwise, all experiments were conducted at least twice, with at least 5 mice per group. Results were expressed as mean ± SD and analyzed for differences between 2 groups using a 2-tailed t test for assuming equal variances, with a P value of less than 0.05 deemed significant. One-way ANOVA test was used for comparing the results of 3 or more groups.

**Results**

**Curcumin inhibits tumor growth in a gastric cancer xenograft model**

We first investigated the effect of curcumin on gastric cancer growth in a MKN-45 cell xenograft model. Treatment with curcumin in the diet delayed tumor growth in the 4-week experimental period (Fig. 1A) and reduced tumor weight by 46.3% at the end of the experiment (P < 0.05; Fig. 1B). The treatment with curcumin by intraperitoneal injection significantly inhibited the established tumor growth (Fig. 1C) and reduced tumor weight by 49.2% as compared with the control group at the end of the experiment (Fig. 1D). No differences in food intake, body weight, and pathologic alterations in major organs were observed between the curcumin-treated groups and control groups.

![Figure 1. Curcumin inhibits gastric cancer xenograft tumor growth. A and B, treatment with curcumin in the diet delayed tumorigenesis in athymic nude mice (A) and reduced tumor weight at the termination of the experiment (B). C and D, treatment with curcumin by intraperitoneal injection inhibited the established tumor growth (C) and reduced tumor weight (D). Data are the means ± SD of tumor weight (n = 5). * P < 0.05, compared with control group. i.p., intraperitoneal.](image-url)
Curcumin inhibits the mobilization and accumulation of MDSCs in a xenograft model

To investigate the mechanism by which curcumin inhibits tumor growth, we examined the effect of curcumin on MDSCs. We found that MDSCs were more abundant in the spleens of tumor-bearing mice than in tumor-free mice, consistent with a previous report (2). The percentages of MDSCs in the spleen (Fig. 2A and B) and blood (Fig. 2C) were significantly decreased in the tumor-bearing mice that were treated with curcumin in the diet (14 days) or by intraperitoneal injection (5 days) compared with the control groups. The tumor volumes in each group were not significantly different at this time point.

Curcumin inhibits allograft tumor growth and the accumulation of MDSCs

To determine whether curcumin inhibits tumor growth in an allograft model, BALB/c mice injected with CT26 cells were treated with 2% curcumin in the diet for 4 weeks or by daily intraperitoneal injection of curcumin for 3 weeks. Both treatments significantly decreased tumor volume (Fig. 3A), tumor weight (Fig. 3B), and the percentages of MDSCs in the spleens and tumor tissues (Fig. 3C; Supplementary Fig. S1A and S1B), as compared with the control groups.

Next, we investigated whether the decrease in MDSC percentages in spleens was due to a direct proapoptotic effect of curcumin. We found that the apoptotic rate of splenic MDSCs from the tumor-bearing mice was significantly lower compared with the naive mice, indicating that MDSCs in the tumor-bearing mice may have a prolonged lifespan in vivo (data not shown). However, curcumin treatment of the tumor-bearing mice for 5 days significantly increased the early apoptosis of the freshly isolated splenic MDSCs (Fig. 3D). Consistent with the results, treatment with curcumin in the diet also increased apoptosis of MDSCs in the spleen (35.3% vs. 21.2% of the control). The data suggest that curcumin induces apoptosis of MDSCs in vivo.

To show that curcumin treatment has an effect on the activation of MDSCs in vivo, the splenic MDSCs from the curcumin-treated tumor-bearing mice were restimulated by
CC-M. The MDSCs from the curcumin-treated mice produced lower amounts of IL-6 in response to CC-M than the MDSCs from the DMSO-injected tumor-bearing mice (Supplementary Fig. S1C), indicating that in vivo curcumin treatment has a subsequent effect on MDSC activation ex vivo. Treatment of curcumin in the diet or by intraperitoneal injection significantly reduced the levels of murine IL-6 in the tumor tissues (Fig. 3E) and serum (Supplementary Fig. S1D).

Curcumin induces the polarization of MDSCs toward a M1-like phenotype

We determined the effect of curcumin on the subsets of MDSCs—granulocytic MDSCs (CD11b<sup>+</sup>Ly6G<sup>+</sup>Ly6C<sub>low</sub>) and monocytic MDSCs (CD11b<sup>+</sup>Ly6G<sup>-</sup>Ly6C<sub>high</sub>; ref. 1). We found that curcumin treatment decreased the percentage of granulocytic MDSCs, but not monocytic MDSCs in the tumor tissues (Fig. 4A; Supplementary Fig. S2A). Similar results were obtained from the spleen tissues (Supplementary Fig. S2B). The results indicate that curcumin mainly reduces the accumulation of granulocytic MDSCs in tumor-bearing mice.

Recent studies suggest that MDSCs may differentiate into tumor-associated macrophages expressing alternative macrophage activation (M2) markers (33). We found that curcumin treatment increased the percentage of CD11b<sup>+</sup>F4/80<sup>+</sup> monocytes/macrophages in the tumor tissues compared with DMSO treatment (Fig. 4B; Supplementary Fig. S2C). Then, we asked whether curcumin could induce the differentiation of MDSCs toward M1-like...
monocytes/macrophages. Curcumin treatment increased the percentage of CCR7$^+$ (M1 marker) cells and decreased the percentage of dectin 1$^+$ (M2 marker) cells (Fig. 4C; Supplementary Fig. S3A) among MDSCs population of tumor tissues (34, 35), and increased the mean fluorescence intensity (MFI) of CCR7 and decreased the MFI of dectin 1 in MDSCs from curcumin-treated mice (Fig. 4D). The data suggest that curcumin promotes the polarization of MDSCs toward M1-like phenotype in vivo. Furthermore, treatment with curcumin in vitro increased the percentage of CCR7$^+$ cells (Fig. 4E) and reduced the percentage of dectin 1$^+$ cells in sorted splenic MDSCs (from tumor-bearing mice; Fig. 4F). Real-time PCR showed that curcumin treatment increased mRNA expressions of iNOS (Supplementary Fig. S3B) and COX-2 (Supplementary Fig. S3C; M1 marker) in MDSCs and decreased expression of YM-1 (M2 marker; Supplementary Fig. S3D; refs. 34, 35). Western blot showed that curcumin upregulated expression of COX-2 protein in MDSCs (Fig. 4G). The data suggest that curcumin induces the polarization of MDSCs toward M1-like phenotype in vivo and in vitro.

**Curcumin inhibits the interaction of MDSCs with cancer cells**

Next, we investigated the interaction of cancer cells with MDSCs. The EGFP$^+$ MKN-45 cells were cocultured with primary MDSCs isolated from tumor-bearing mice. The results showed that curcumin treatment inhibited the interaction of MDSCs with cancer cells, as evidenced by decreased level of CCR7$^+$ and dectin 1$^+$ cells (Fig. 4C and D). These findings support the hypothesis that curcumin may have therapeutic potential for cancer treatment by targeting MDSCs.
with splenic MDSCs isolated from the tumor-bearing mice. There was a 50% increase in the number of MKN-45 cells in the coculture system compared with cancer cells that were cultured alone (Fig. 5A). The merged images indicate that the colonies originated from EGFP⁺ MKN-45 cells (bottom). Quantification of colonies of cancer cells were shown in (B). The production of IL-6 by MDSCs and its inhibition by curcumin are shown in (C). The data are means ± SD of 3 independent experiments (*, P < 0.05, #, P < 0.01, compared with DMSO treatment).

Curcumin inhibits MDSCs and tumor growth. The EGFP⁺ MKN-45 cells and splenic MDSCs were cultured alone or cocultured in the absence or presence of curcumin. A, representative images (magnification 200×) were taken 48 hours after curcumin treatment (25 μmol/L; top). The merged images indicate that the colonies originated from EGFP⁺ MKN-45 cells (bottom). The cocultured cancer cells formed a number of large colonies, whereas MDSCs or gastric cancer cells cultured alone did not form any colony (Fig. 5A and B). Fluorescence microscopy further confirmed that only EGFP⁺ MKN-45 cells formed colonies (Fig. 5A, bottom panel). The results indicate that cancer cells acquire a growth advantage via interactions with MDSCs.

Then, we examined the effect of curcumin on the interactions of MDSCs with cancer cells. The curcumin treatment inhibited proliferation of both MDSCs and cancer cells alone or cocultured (Fig. 5A), but the inhibitory effect of curcumin on the cocultured cells was less extensive compared with those cultured alone, suggesting that the coculture of MDSCs with cancer cells produces a
survival advantage for both types of cells. However, the treatment with curcumin markedly reduced colony growth of gastric cancer cells in a coculture system (Fig. 5A and B). In this coculture system, curcumin (12.5 μmol/L) decreased survival of MKN-45 cells by 60% ± 5.4%, but the apoptotic rate of MKN-45 cells was only induced by 25% ± 3.2%, suggesting that the decreased cell growth by curcumin is largely due to abolishing the growth advantage of cancer cells acquired from the interaction with MDSCs.

To investigate the effect of gastric cancer on MDSC activation, we determined the number of MDSCs and the level of murine IL-6 in the cell supernatant. When cocultured with gastric cancer cells, the number of MDSCs increased 1.8-fold, and the level of murine IL-6 secreted by MDSCs increased 9-fold in the coculture cell supernatant (Fig. 5C), suggesting that human gastric cancer cells activate mouse MDSCs. Moreover, the treatment with curcumin significantly reduced murine IL-6 secretion from MDSCs in this coculture system (Fig. 5C). The data indicate that curcumin inhibits cancer cell–induced expansion of MDSCs and secretion of IL-6.

Curcumin suppresses the activation of Stat3 and NF-κB in MDSCs

To further investigate the mechanisms by which curcumin inhibits the interaction of MDSCs with cancer cells, we determined the effect of curcumin on Stat3 and NF-κB in MDSCs. Curcumin significantly inhibited IL-1β–stimulated production of IL-6 by MDSCs (Fig. 6A). To determine whether curcumin inhibited MDSC activation through the NF-κB pathway, we isolated EGFP+ splenic MDSCs from IL-1β; NF-κBEGFP transgenic mice (7), in which EGFP expression was controlled by a cis-element of the NF-κB pathway. The splenic MDSCs, treated with curcumin for 48 hours, were analyzed by FACS. Curcumin inhibited expansion of MDSCs. Bone marrow–derived cells from tumor-free mice were cultured for 3 days in NM, CC-M, or MF-M in the absence or presence of curcumin. The percentages of Gr-1+CD11b+ cells were determined by FACS. D and E, the splenic MDSCs isolated from tumor-bearing mice were cultured in indicated media in the absence or presence of curcumin (12.5 μmol/L) for 48 hours. Protein levels were determined by Western blot (D), and IL-6 level in the cell supernatant was measured by ELISA (E). The data are means ± SD of 3 independent experiments (*, P < 0.01).
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promoter. The expression of EGFP indicates the activation of cis-element of the NF-κB promoter. Treatment with curcumin reduced the number of EGFP+ cells (Fig. 6B), suggesting that curcumin inhibits the activation of the NF-κB promoter. The exosomes released from tumor cells have been shown to induce expansion of MDSCs and IL-6 production in MDSCs in a Toll-like receptor 2/Stat3-dependent manner (36). Myofibroblasts are a major type of stroma cells in tumor microenvironment (31). We found that CC-M and MF-M induced expansion of MDSCs from naive mice and that curcumin inhibited CC-M- and MF-M–induced expansion of MDSCs (Fig. 6C). Furthermore, CC-M and MF-M increased the expressions of p-Stat3 and p-p65 (Fig. 6D) and production of IL-6 in MDSCs (Fig. 6E). Curcumin inhibited the expressions of Stat3, p-Stat3, p65, p-65, and survivin (Fig. 6D) and reduced IL-6 levels in MDSCs (Fig. 6E).

Discussion

MDSCs play important roles in cancer development and progression (1, 8). In this study, we showed that treatment with curcumin in the diet or by intraperitoneal injection inhibited tumor growth and the mobilization and activation of MDSCs and polarized MDSCs toward M1-like phenotype in both BALB/c mouse allograft and athymic nude mouse xenograft models. Curcumin suppressed Stat3 and NF-κB activation and reduced IL-6 production in MDSCs. Our results reveal new mechanisms for the antitumor actions of curcumin.

MDSC accumulation in the tumor microenvironment promotes tumor growth (7). Curcumin in the diet or by intraperitoneal injection was able to inhibit MDSC accumulation in the spleen and in the tumor bed, mainly reducing the accumulation of PMN-MDCs. IL-6 has been reported to induce the expansion and mobilization of MDSCs (10). Curcumin treatment significantly reduced the level of murine IL-6 in the serum and tumor tissues of mice and induced MDSCs apoptosis in vivo. Thus, the reduction of MDSC accumulation by curcumin may be due to inhibition of MDSC expansion and induction of apoptosis of MDSCs.

An important finding in this study is that curcumin inhibits the activation of Stat3 and NF-κB in MDSCs. The activation of Stat3 and NF-κB regulates the expression of antiapoptotic, pro-proliferative, and immune response genes (37–40). Cytokines, such as IL-6, which are induced by NF-κB activation in stromal cells, often activate Stat3 in both malignant cells and stromal cells (37). Inhibition of NF-κB and Stat3 activation is becoming effective therapeutic strategies for cancer (39, 41, 42). Stat3 has been shown to regulate MDSC expansion (11). We found that both Stat3 and NF-κB were activated in MDSCs, resulting in increased levels of IL-6, and the activation was inhibited by curcumin. Consistent with the in vitro results, treatment of mice with curcumin decreased the level of IL-6 in the tumor tissues and serum. Our results are consistent with recent reports showing that curcumin decreased the expressions of Stat3 and NF-κB in cancer cells (43) and the peripheral blood monocytes of cutaneous T-cell lymphoma patients (28). Thus, inhibition of the activation of Stat3 and NF-κB in MDSCs may be an important antitumor mechanism of curcumin.

Another important finding is that curcumin can inhibit the interaction of MDSCs with gastric cancer cells. Previous studies of MDSCs have focused on their interaction with T-cells (1). Although some studies have shown that tumor cell–secreted exosomes induce expansion of MDSCs, the direct effect of MDSCs on tumor cells has not been sufficiently studied (36, 44, 45). We found that the MDSCs stimulated gastric cancer cell proliferation and colony formation; in return, the gastric cancer cells activated MDSCs to secrete IL-6. Notably, curcumin treatment significantly inhibited cancer cell proliferation and colony formation and decreased the secretion of murine IL-6 by MDSCs in this coculture system. On the basis of ours and other results, we propose that cancer cell–secreted factors (such as exosome and IL-1β) activate Stat3 and NF-κB signaling pathways in MDSCs, resulting in enhanced production of IL-6. IL-6 secreted by MDSCs then activates Stat3 pathway in cancer cells, resulting in promoting cancer cell proliferation. Curcumin inhibits the Stat3 and NF-κB pathways in MDSCs and reduced IL-6 production, resulting in the inhibition of IL-6/Stat3 pathway in cancer cells and tumor growth. Thus, curcumin disrupts the interaction of cancer cells with MDSCs through inhibition of IL-6/Stat3 and NF-κB pathways.

A new finding in this study is that curcumin polarizes MDSCs toward a M1-like phenotype both in vivo and in vitro. M1 macrophages promote the cytotoxicity and inhibit tumor growth, whereas M2 macrophages induce immune suppression and tumor progression (46). M1 macrophages express some characteristic molecules such as iNOS, COX-2, CCR7, and proinflammatory cytokine (TNF-α and IL-1β), and M2 macrophages express arginase-1, YM-1, mannose receptor, detcin 1, and cytokine IL-4 and IL-10 (34, 35, 46). Curcumin increased expressions of iNOS and COX-2 and decreased expression of YM-1 in MDSCs. Thus, the polarization of MDSCs toward a M1-like phenotype may be a mechanism of antitumor actions of curcumin. One potential mechanism underlying polarization of MDSCs to M1-like phenotype is that curcumin inhibited Stat3 pathway. Stat3 activation has been shown to be associated with M2 macrophage polarization (46, 47). Stat3 may be activated by M2-macrophage-polarizing cytokines such as IL-10 and IL-4 (46, 47). Our results are consistent with a previous report that docetaxel and curcubatin I, a Stat3 pathway inhibitor, inhibited p-Stat3 activation, resulting in induction of differentiation of MDSCs toward M1-like phenotype (48). However, further studies are needed to define the detailed mechanisms of curcumin-polarizing MDSCs to M1 macrophages.

In summary, our results have shown that curcumin inhibits the activation of MDSCs, induces the differentiation of MDSCs, interferes with the interaction between
MDSCs and cancer cells, and suppresses tumor growth. These results reveal a new mechanism for curcumin to inhibit tumorigenesis and suggest that targeting MDSCs may be a promising strategy for cancer prevention and therapy.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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