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ORIGINAL ARTICLE

Down-regulation of MFG-E8 by RNA interference combined with doxorubicin triggers melanoma destruction

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Abstract The pathogenic mechanism of malignant melanoma involves the dynamic interplay of transformed cell and normal host cell, but cancer treatments always target each partition separately. In the tumor microenvironment, milk fat globule epidermal growth factor-8 (MFG-E8) is a secreted glycoprotein highly expressed in the vertical growth phase of melanoma, leading to tumor progression through coordinated $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrin signaling in tumor cells and host cells. Doxorubicin (Dox) is one of the most widely used antitumor drugs against a lot of solid tumors, including melanoma. In this work, Dox was used to combine with down-regulation of MFG-E8 by RNA interference (RNAi) in order to determine the synergistic effect of the antitumor activity in vivo. And the possible mechanisms were investigated. Results showed that combination group (MFG-E8 RNAi plus Dox) could inhibit the growth of melanoma more effectively than monotherapy or control groups. We found that the combination treatment induced more tumor cell apoptosis and inhibited more neovascularization than other groups. Moreover, this combination treatment attenuated $CD4^+$ $CD25^+$ $Foxp3^+$ Treg cells in tumor-infiltrating lymphocytes compared with other groups. Our findings suggested that MFG-E8 down-regulation enhanced the antitumor function of chemotherapy through coordinated cell apoptosis and immune-mediated mechanisms, which might be a feasible way for cancer therapy.

Keywords MFG-E8 · RNAi · Doxorubicin · Melanoma cancer · Gene therapy

Abbreviations

MFG-E8	Milk fat globule epidermal growth factor-8
Scr	Scrambled control si-RNA
Dox	Doxorubicin
TRAIL/Apo2L	Tumor necrosis factor-related apoptosis-inducing ligand
VEGF	Vascular epidermal growth factor

Introduction

Malignant melanoma is a common and aggressive skin tumor with an overall mortality rate of about 14.5 % [1], and the prognosis for early melanoma is favorable with a 5-year overall survival of >90 %, late melanoma decreases the overall survival rate by 10–46 % [2]. Malignant melanoma is often associated with early metastasis and a high-level of resistance to current therapies [3]. It is a serious public health matter worldwide for the limited treatment and unfavorable prognosis [4]. Therefore, the development of an effective therapy for malignant melanoma is required.

Milk fat globule epidermal growth factor-8 (MFG-E8) is a secreted glycoprotein earliest discovered as a membrane element of MFGs [5]. MFG-E8 is expressed in a variety of cells including macrophages, dendritic cells, myoepithelial cells and endothelial cells, retinal and intestinal epithelial

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cells [6], and it is also expressed at high levels in many tumor types [7, 8], including melanoma. In malignant melanoma, MFG-E8 expression is increased in cancer cells and/or infiltrating myeloid elements in the vertical growth phase, promoting tumor progression through coordinated $\alpha_v\beta_3$ integrin signaling in the tumor microenvironment [9, 10]. Under steady-state conditions, GM-CSF triggers MFG-E8 expression in macrophages and dendritic cells, enabling the efficient uptake of apoptotic cells, the maintenance of $Foxp3^+$ regulatory T cells, and the suppression of autoreactive Th1 and Th17 cells. Under the conditions of stress, however, Toll-like receptor agonists or necrotic cells down-regulate MFG-E8 levels, whereupon GM-CSF elicits $CD4^+$ and $CD8^+$ effector T cells through an MFG-E8 independent way. Thus, the presence of MFG-E8 in the tumor microenvironment might modulate the functions of GM-CSF during carcinogenesis and skew GM-CSF activity toward disease promotion rather than inhibition [11]. Consistent with this phenomenon, $\alpha_v\beta_3$ integrin inhibitor can reduce the bone turnover in the patients with hormone-refractory prostate cancer and bone metastases although serum PSA was increased [12]. In a murine melanoma model, MFG-E8 enhances tumorigenicity and metastatic capability by Akt- and Twist-dependent ways [9]. In addition, MFG-E8 augments melanoma cell resistance to apoptosis, induces an epithelial-to-mesenchymal transition, enhances melanoma cell survival, stimulates invasion and angiogenesis, with augmenting vimentin and N-cadherin and reducing E-cadherin [13–15], and also contributes to local immunosuppression by increasing $Foxp3^+$ Treg cells.

As noted above, MFG-E8 is expressed at high levels in melanoma and acts as a potent tumor promoter in the development of melanoma; so, MFG-E8 might be served as a new target for cancer therapy. In contrast to conventional cancer treatments, which always target either tumor or host, MFG-E8 antagonist may affect these two partitions [11]. The RNA interference (RNAi) pathway is an effective and specific pathway involved in post-transcriptional gene silencing, widely used for manipulating biological systems [16]. Thus, down-regulation of MFG-E8 by RNAi is a promising pathway target for the therapies of melanoma. Moreover, chemotherapy plays an important role in the treatment of melanoma, and doxorubicin (Dox) is one of the most widely used antitumor drugs because it presents considerable activity against a set of solid tumors, including melanoma [17]. In clinical trials, Dox is a conventional chemotherapeutic drug to melanoma [18]; unfortunately, as for antitumor drugs in general, tumors are often resistant either from the beginning or become so after chemotherapy [18]. We know that Dox in the clinical application has several side effects. The most prominent acute side effect is hematological. Nausea and vomiting, hair loss and oral mucositis represent common side effects [19]. And cardiac toxicity is the common side effect, mainly consist of dilatation of sarcoplasmic reticulum, myofibrillar loss and interstitial fibrosis [20]. Actually, shRNA knockdowns of MFG-E8 can increase tumor cells' sensitivity to small molecule inhibitor of receptor tyrosine kinases and cytotoxic agents in vitro [9, 21]. Based on these results, we conjectured that MFG-E8 down-regulation by RNAi might inhibit tumor development in some complementary ways. In this paper, we show that small interfering RNA (siRNA) interference of MFG-E8 cooperating with Dox to complete sustained control of established mouse melanoma. And a preliminary study of mechanisms was assayed.

Materials and methods

Materials

Female C57BL/6 mice (all 6–8 weeks of age) were obtained from the Beijing HFK Bioscience Co. Ltd. (HFK). The B16 cell line was purchased from American Type Culture Collection (Rockville, MD, USA). The cells were maintained in complete culture media in RPMI-1640 (Sigma-Aldrich, Shanghai, Trading Co. Ltd.) containing 10 % heat-inactivated fetal bovine serum under the condition of 5 % CO_2 in an incubator at 37 °C. The siRNA against mouse MFG-E8 (Si-m-MFGE8) and MFG-E8 scrambled control siRNA were purchased from Guangzhou Ribo Bio Co. Ltd. The liposome (DOTAP-DOPE) complexes were prepared by our laboratory and incubated with the siRNA at room temperature 20–30 min; the mixture was intratumorally given to the animals. Dox was obtained from West China hospital, Sichuan University. Anti-CD31 was purchased from PharMingen, San Diego, CA. Anti-MFG-E8 was obtained from MBL International. HRP-conjugated anti-hamster IgG was purchased from Santa Cruz Biotechnology, Inc. Treg flow kit (CD4, CD25, Foxp3) was purchased from eBioscience company.

In vivo treatment

To establish s.c. tumors, female C57BL/6 mice (6–8 weeks of age) were injected with 5×10^5 B16 tumor cells in the right flank. Four days after inoculation of tumor cells, the 50 mice were randomly split into five groups and each group has ten animals: (1) untreated (NS), (2) DOX, (3) scrambled control si-RNA (Scr) plus Dox (Scr + DOX), (4) si-m-MFG-E8 (Si-mfge8), (5) si-m-MFG-E8 and Dox combination (COM). The siRNA-coding oligos opposed to mouse MFG-E8 were designed using BLOCK-i T RNAi designer (Invitrogen) and checked out specificity by vast search against the mouse genome. The sequence used was as follows: ACAAGACATGGAACCTGCGTGCTTT, the

siRNA sequence and scrambled control sequences do not match any known murine cDNA [9]. SiRNA and scrambled control siRNA were mixed with liposome and injected intratumorally with the dose of 10 μg once every 3 days commencing on day 7 (seven times in total). Dox was injected intraperitoneal 5 mg/kg in a volume of 200 μl per mouse and was carried out in the 4th, 11th, 18th and 25th days (four times in total). The tumor measured by vernier calipers: the shortest axis (a) and the longest axis (b) of tumor we measured every 3 days. The tumor volumes were calculated using the formula (tumor volume $= a^2 \times b \times 0.52$).

Flow cytometry

On day 28 after tumor cell inoculation, mice were killed (three mice of each group). Metastasizing lymph nodes in the right groin were harvested from the mice, sliced into tiny pieces with scissors, mechanically dispersed in 3–5 ml cold RPMI medium and adjusted to a concentration of 1×10^5 cells in 100 μl of PBS. Lymphocyte suspension was incubated with anti-CD4 and CD25 mAbs, washed and then stained with anti-Foxp3 antibody according to the manufacturer's protocol of Treg cell flow kit (eBioscience). The frequency of each sample was determined by flow cytometry. Cells were taken by a FACSCalibur flow cytometry (BD Biosciences), and data were analyzed with Flow Jo software 7.6.

Immunohistochemistry

Tumors were harvested from the mice at the end of the experiment, three tumors of each group for stain, and each tumor was bisected, one-half was fixed in 10 % formaldehyde solution for 48 h and then processed for paraffin embedding. The remaining halves were snap frozen for immunohistochemical staining. And other three tumors of each group were put into liquid nitrogen for Western blot. Before immunohistochemical staining, we bleached melanin by dilute hydrogen peroxide [22]. Frozen sections for each of the 5 groups were stained for CD31. Rabbit anti-rat IgG secondary biotinylated antibody was applied with a standard streptavidin-peroxidase label and DAB substrate (DAKOcytomation, Carpinteria, CA). CD31-positive (brown) cells in the tumor vessels were quantified by microscopy (original magnification 200×) at least 5 random fields and were calculated as relative micro-vessel density.

TUNEL assay

Apoptosis was estimated by using the DeadEnd™ Fluorometric TUNEL System (promega, USA). A total of 5 μm paraffin-embedded tissue sections were prepared by dewaxing and hydration, then, fixed with 4 % formaldehyde for 15 min, washed in PBS and permeabilized with 20 μg/ml proteinase K for 10 min at room temperature. A positive control was produced by adding 1 μg/μl DNase I in PBS/1 mM MgSO4. The reaction mixture (50 μl) contained equilibration buffer 45 μl, TdT 5 μl labeling reaction mix and TdT enzyme 1 μl was added to each section, reacted for 1 h at 37 °C and then washed and sealed with 50 % glycerin. Eventually, these sections were analyzed using fluorescence microscopy, and the apoptotic index was defined as the percentage of apoptotic nuclei counted per 1,000 neoplastic nuclei. The fields of tumor were chosen randomly at 200× magnification.

Western blot

Tumor tissues in the liquid nitrogen were taken out and a moderate amount of tissues of each group was put into the mortar, kept pouring liquid nitrogen into the mortar and fully ground and homogenized in 2 ml of the buffer containing appropriate protease inhibitors. We centrifuged the crude homogenized at 100,000×*g* for 60 min at 4 °C, transferred the supernatant to another tube, then quantified the protein and mixed the sample with equal concentration and equal volume with the buffer. Then, we boiled the samples for 5 min and centrifuged. After that, we loaded 10 μl of the sample per lane in a 1-mm-thick SDS–polyacrylamide gel for electrophoresis and blotted the protein to a polyvinylidene difluoride (PVDF) membrane at 100 V for 1 h in a wet transfer system. Then, the membrane was soaked in 5 % skimmed milk (in PBS, PH 7.2) for 1 h at 37 °C to reduce nonspecific binding, and then, we incubated the membrane with primary antibody diluted with PBS, PH 7.2 containing 5 % skimmed milk at 4 °C overnight, washed the membrane with PBS-T (5 % Tween-20 in PBS), incubated the membrane with the HRP-conjugated secondary antibody for 1 h at 37 °C and then washed as before. Then the membrane was incubated with appropriate chemiluminescence reagent and sealed it in plastic wrap, exposed to an X-ray film in a dark room and developed the film as usual. β-actin was used as a loading control to check the integrity of each sample.

Statistical analysis

The statistical significance between values was performed with one-way ANOVA including tests for multiple comparisons using the Excel (Microsoft Corp., Redmond, WA). All the data were presented as the mean ± standard deviation (SD). $p < 0.05$ was considered as statistically significant.

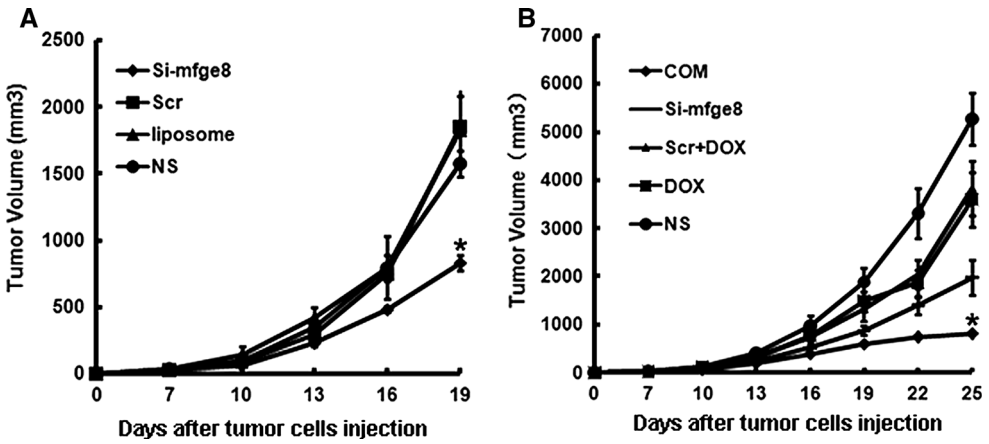

Fig. 1 Tumor growth process in melanoma bearing mice. Mice (ten mice per group) were inoculated with B16 cells and treated. **a** Si-mfge8-RNA, or Scr, or only vector-liposome (lipo) and (or) saline (NS) were injected intratumorally, and the tumor volume growth was slower in Si-mfge8 group than any other three groups. **b** Mice were intratumorally injected with Si-mfge8-RNA, Scr 10 μg/per mice/3 days, and (or) DOX 5 mg/Kg/7 days, and saline at the same time points. Graph shows the treatment with Si-mfge8 or DOX alone inhibiting the growth of tumor, while combination therapy with Si-mfge8 + DOX (COM) results in an obvious suppressing effect of the established B16 melanoma. Results were expressed as average tumor volume ± SD. *$p < 0.05$ versus control

Results

Tumor growth inhibition in establishing murine melanoma

To explore the therapeutic potential of RNAi of MFG-E8, firstly we injected B16 to C57Bl/6 mice. At 7th day inoculation, when tumors were well established, si-MFG-E8-RNA, scrambled control siRNA, only vector-liposome and saline were injected intratumorally once every 3 days, and we found that MFG-E8 RNAi could inhibit tumor growth, while Scr and only vector had no difference with the group of only injection of saline (Fig. 1a). Similar to many chemotherapeutics, Dox leads to serious side effect in long-term use, and tumor cells can resist to chemotherapy [3]. To see whether down-regulation of MFG-E8 could enhance the therapeutic effectiveness of DOX, we examined the synergistic antitumor effects of these two factors on tumor growth in B16 melanoma in C57Bl/6 mice. We found that this combination therapy achieved prolonged tumor control, different with the limited impact of individual agents (Fig. 1b). Collectively, this experiment showed the ability of down-regulation of MFG-E8 by RNAi to enhance the antitumor effects of Dox in the melanoma tumor model.

Down-regulation of MFG-E8 expression by RNAi in tumor

Since the outcome of our combined treatment strategy and RNAi alone have showed the promising effect in suppressing tumor growth, to see the efficiency of gene knockdown by RNAi, Western blotting was done. As shown in Fig. 2, positive siRNA treatment resulted in down-regulation of MFG-E8 expression. The data show that the band of the combined therapy group (COM) and RNAi-alone group (Si-mfge8) were significantly more fragile than other three groups. Nevertheless, the scrambled control siRNA group (Scr + DOX) and Dox-alone group (DOX) had no conflict with the saline group (NS).

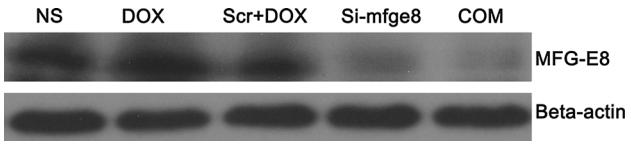

Fig. 2 Expression of MFG-E8 in established murine melanoma tumor. *Western blotting* showed protein expressions of MFG-E8 in combination treatment group (COM) and si-mfge8 group were lower in melanoma tumors than that in any other groups. Representative of three experiments

Combination treatment of MFG-E8 down-regulation and doxorubicin reduces angiogenesis

It is well known that angiogenesis is a significant factor that promotes tumors growth and advancement. Previous researches have shown that MFG-E8 is a potent angiogenic factor in vitro and in vivo [7, 23, 24]. To confirm whether the inhibitory effect of MFG-E8 down-regulation and chemotherapy combination of tumor growth was tied to the reduction of tumor angiogenesis, we calculated the density of vessels inside the tumor mass by immunohistochemical analysis of CD31 expression; CD31 is expressed in vascular endothelial cells. The density of angiogenesis within

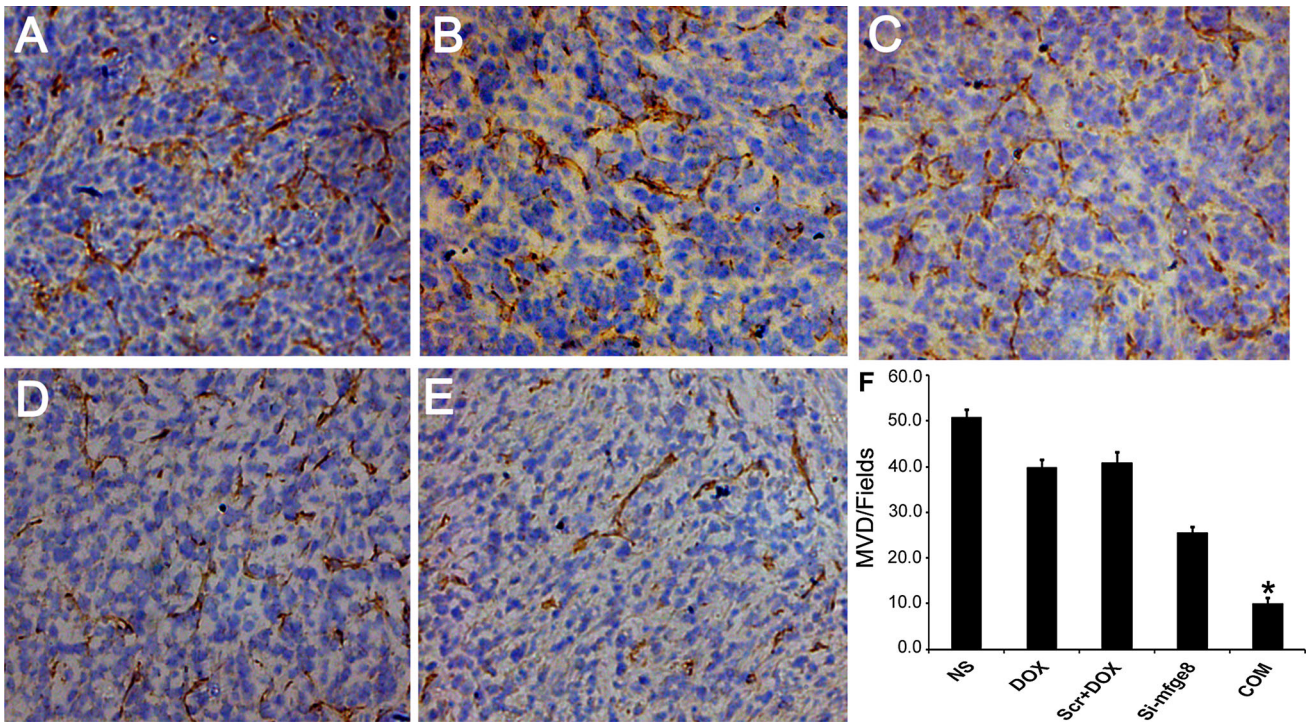

Fig. 3 Inhibition of angiogenesis within tumors estimated by immunohistochemistry with CD31. The frozen sections of tumor tissues were harvested from mice treated with **a** saline (NS), **b** DOX, **c** Scr + Dox (Scr + DOX), **d** Si-mfge8, **e** Si-mfge8 + Dox (COM). Representative sections from tumor tissues are presented. Vessel density was determined by counting the number of the microvessels five random fields in the CD31-stained sections. **f** Combination treatment group displayed a significantly decreased microvessel when compared to the control groups (*$p < 0.05$), and bars represent mean microvessel ± SD in tumor tissues

the tumor was assessed by calculating the number of microvessels by immunolabeling of CD31 in tissue sections. The densities of CD31-positive vascular structures in tumors from mice in the untreated control group (Fig. 3a) were the highest, followed by the Dox group (Fig. 3b), the scramble control si-RNA plus Dox group (Fig. 3c) and MFG-E8 RNAi-alone group (Fig. 3d). Tumor from MFG-E8 RNAi combined with Dox group (Fig. 3e) displayed the least density, and significantly lower ($p < 0.05$) in comparison with the vessels in tumors from mice in the other groups. The combination treatment resulted in obvious inhibition of tumor angiogenesis when compared with the other groups (Fig. 3f).

Combination treatment induces apoptosis in vivo

It is well known that down-regulation of MFG-E8 induces apoptosis in a variety of cancer cells [21, 25]. To determine whether the observed tumor growth inhibition of the combination treatment group was linked to apoptotic cells, we performed terminal deoxynucleotidyl transferase-mediated dUTP nick-end-labeling (TUNEL) assay on the tumors that were harvested at the end of the experiment. The results indicated that few isolated positive nuclei were observed in saline control group (Fig. 4a). Tumor mass from Dox group (Fig. 4b) is more as well as scrambled control + Dox (Fig. 4c). MFG-E8 RNAi-treated group (Fig. 4d) showed an increased apoptosis compared with above three groups. High levels of apoptotic nuclei were exhibited by tumors treated with the MFG-E8 RNAi and Dox combination therapy (Fig. 4e). Data were shown as the mean apoptotic index ± SDs of tumor cells as a percent normalized to apoptotic index of cancer cells (Fig. 4f).

Combinatorial therapy inhibits Treg cells in the tumor microenvironment

As the immune regulation is considered to be one of the most important ways to control tumor growth and progression, it is vital to obtain a better understanding of the interactions between the immune system and tumorigenesis. Previous researches showed that MFG-E8 inhibited vaccine-stimulated tumor immunity by the induction of Treg cells [9, 21]. Since our combination treatment shows a promising effect in suppressing tumor growth, we investigated the combined effect of Dox treatment and MFG-E8 RNAi therapy on the Treg cells that were isolated from tumor-infiltrating lymphocytes (TILS) from B16 mice. The flow cytometry data showed that the absolute number of Treg cells was significantly lower in mice receiving MFG-

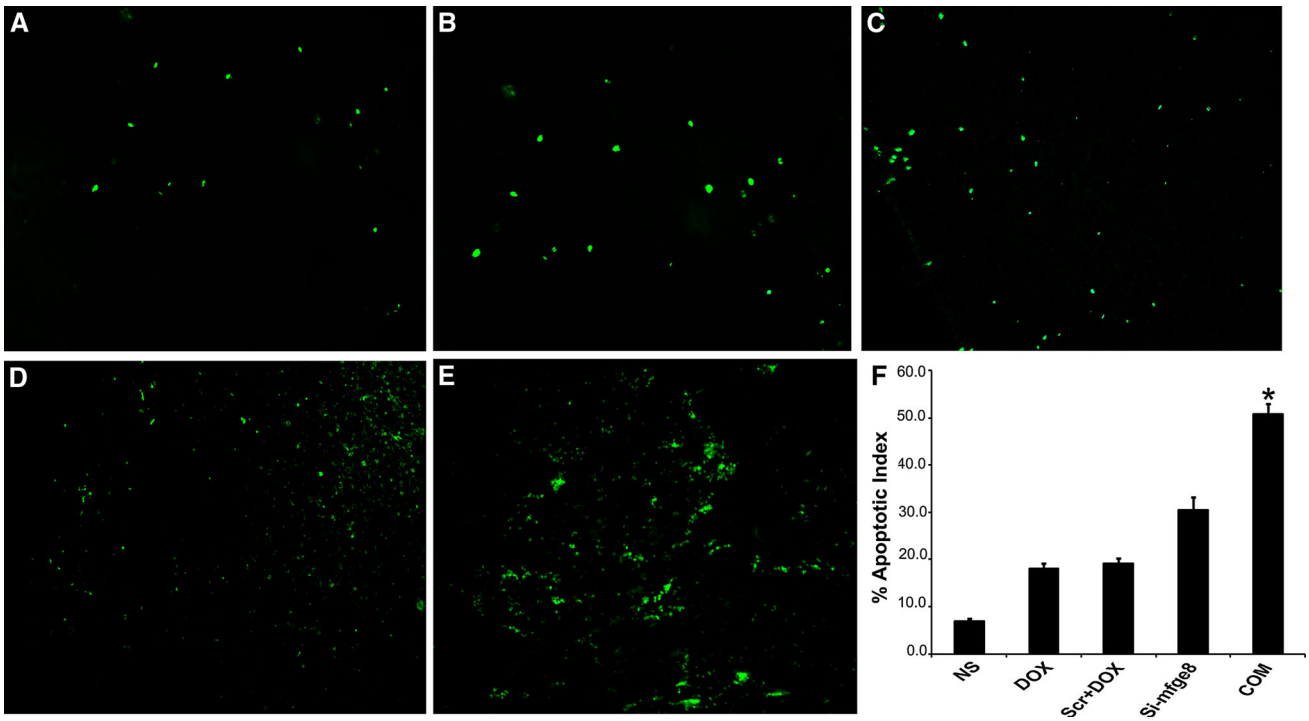

Fig. 4 Assays for the detection of apoptosis. Apoptotic tumor cells within tumor tissues were detected by TUNEL assays in accordance with the manufacture's instruction. Representative sections from tumor tissues are presented; **a** saline (NS), **b** DOX, **c** Scr + Dox (Scr + DOX), **d** Si-mfge8, **e** Si-mfge8 + Dox (COM). **f** The treatment with Si-mfge8 + DOX showed an apparent increment of apoptotic cells within tumor tissues versus controls (*$p < 0.01$). *Bars* represent the mean apoptotic index ± SD of tumor cells

E8 RNAi combined with Dox therapy (Fig. 5e) compared with mice from the other four groups. Meanwhile, the total number of Treg cells from RNAi treated alone (Fig. 5d), Dox alone (Fig. 5b), is similar to that of the lymphocytes from the scrambled control combined with Dox group (Fig. 5c), and the untreated control group (Fig. 5a) is the highest. Thus, the combination therapy resulted in synergistic inhibition of Treg cells when compared with the controls (Fig. 5f).

Discussion

Although definite evidence demonstrates that dynamic interaction between tumor cells and normal host cells is very important to carcinogenesis [26, 27], and evidence indicates that the antitumor strategy through a combination treatment may be more efficacious than monotherapy [11], almost all the cancer therapies mainly direct at individual factors. Now RNAi of tumor relevant protein serves as rudiment of reasonable and new treatment that resists major pathogenic mechanisms in tumor cells and host cells [28]. Like blockade of vascular epidermal growth factor (VEGF) by antibody or RNAi, this treatment obtains important clinical benefits, but most patients can only achieve partial responses and eventually die of progressive disease caused by drug-resistant variants [11]. To address this issue, our study developed a combination therapy, MFG-E8 RNAi plus Dox that successfully controlled B16 melanoma cancer growth.

MFG-E8 promotes tumor progression by coordinating $\alpha_v\beta_3$ integrin signaling in cancer cells, vascular factors and infiltrating myeloid cells. We found that, although the treatment of MFG-E8 RNAi alone showed mild tumor decrease and immune stimulation, the combination of Dox and MFG-E8 RNAi achieved sustained regressions. A key component of this synergy is the ability of down-regulation of MFG-E8 decrease tumor cell resistance to chemotherapy. An additional mechanism by which MFG-E8 down-regulation might increase tumor cell killing, possibly related to a more powerful inhibition of tumor angiogenesis; MFG-E8 is the key to VEGF-induced angiogenesis [7, 23, 29–31]. Thus, down-regulation of MFG-E8 could inhibit angiogenesis. As we expected, the combination treatment significantly inhibited tumor vessels growth than other four groups.

Previous studies have demonstrated that MFG-E8 as a bridge of integrins on macrophages and phosphatidylserine and phosphatidylethanolamine residues on apoptotic cells, which set up intercellular interactions that enhance the engulfment of apoptotic cells by macrophages [25, 32, 33]. Furthermore, tumor necrosis factor (TNF)-related

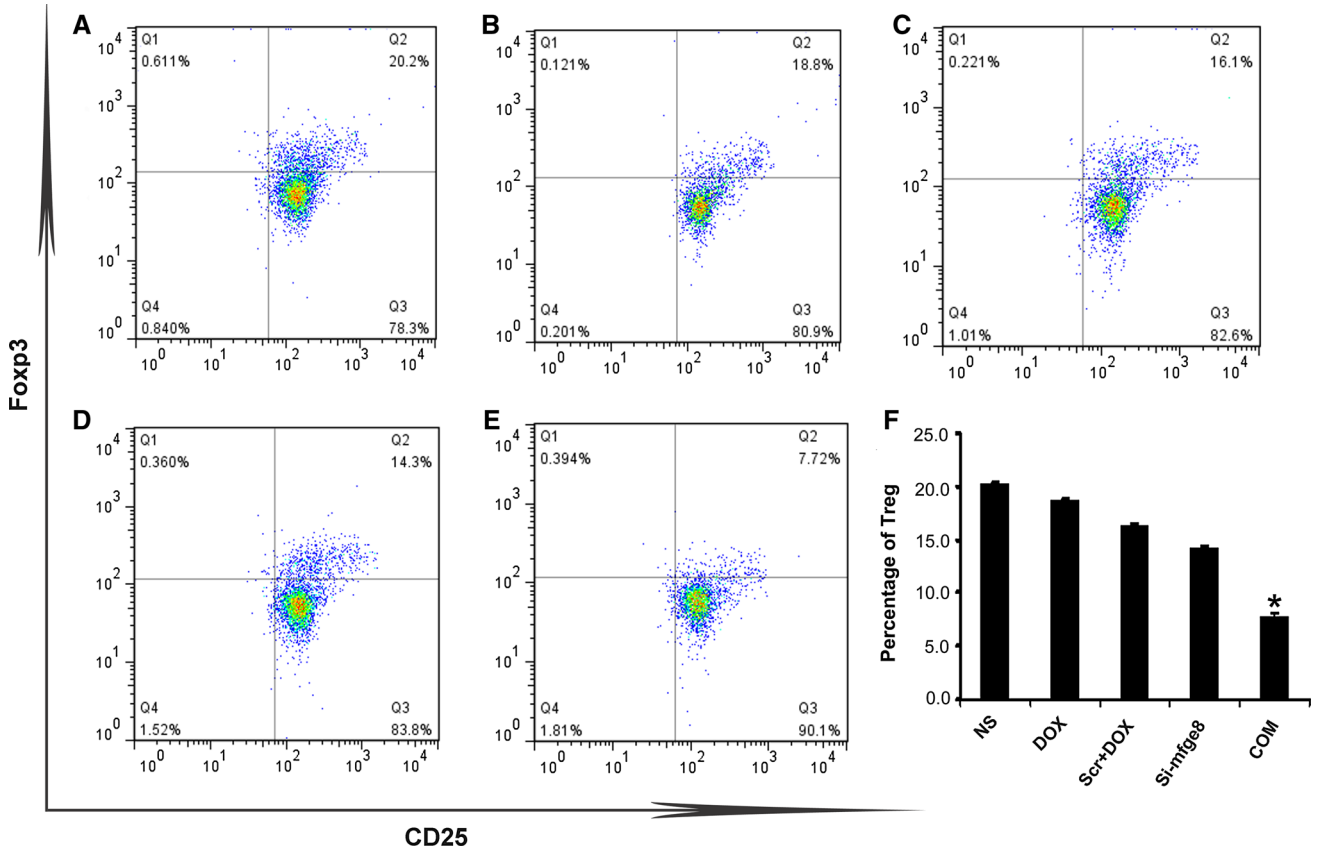

Fig. 5 FCM analysis of Treg cells in TILS. TILS were harvested from mice bearing B16 tumors 28 days after the indicated treatment. The TILS were gated as $CD4^+CD25^+$ T cells, and assayed for CD25 and Foxp3 with flow cytometry (percentages are shown). Representative stainings are presented, **a** saline (NS), **b** DOX, **c** Scr + Dox (Scr + DOX), **d** Si-mfge8, **e.** Si-mfge8 + Dox (COM). **f.** The mean ± SD for three mice per group are shown. FCM test was independently performed three times. The treatment with Si-mfge8 + DOX showed an apparent decrement of Treg cells in TILS versus controls ($*p < 0.05$)

apoptosis-inducing ligand (TRAIL/Apo2L) is a member of the TNF gene superfamily that induce apoptosis [34]. And previous studies have shown that Dox induce cell death, which is linked to DNA damage, oxidative mitochondrial damage and nuclear translocation of p53 [35]. Meanwhile, pretreatment with Dox is sufficient to sensitize cells to TRAIL/Apo2L [36]. In addition, MFG-E8 down-regulation also enhances cross-presentation of dying tumor cells and dendritic cells efficiently [11]. Consistent with these results, we found that MFG-E8 down-regulation combined with Dox induced more apoptosis in tumor tissues than other groups. It is helpful to conjecture that this apoptosis response may promote long-term protection against tumor progression and TRAIL/Apo2L might be a novel related mechanism.

Upon the consequence of tumor small amplitude decrease with Dox treatment, MFG-E8 down-regulation is conducive to build an immumitaet tumor microenvironment. This outcome reflected the dual ability of MFG-E8 down-regulation to resist $\alpha_v\beta_3$ integrin-mediated immune suppression. And recent studies of down-regulation of MFG-E8 could stimulate T cell immunity by reducing the numbers of $Foxp3^+$ Tregs and increasing $CD4^+$ and $CD8^+$ effector T cell activation and function [11], perhaps through the enhancement of Twist and the activation of STAT-3 [9], meanwhile receded NF-KB pathway [37]. In addition, recent research has shown that some chemotherapies promote immunogenic cell death induced by the cell releasing calreticulin [38–40]. Dox additionally induces the DNA damage response to the increasing tumor cell expression of NKG2D ligands and then stimulates $CD8^+$ T cells and NK response [41]. Consistent with these results, we found that MFG-E8 down-regulation combined with Dox limited $Foxp3^+$ Treg cells in tumor-infiltrating lymph node. It is helpful to conjecture that this T cell response may inhibit the appearance of drug-resistant tumor cells and promote long-term protection against tumor progression. According to this notion, previous researches and clinical studies have displayed that a high ratio of effector T cells to Treg cells is very important to sustain tumor destruction [42–44].

In conclusion, compared with controls, combination treatment of MFG-E8 down-regulation and Dox showed an

apparent antitumor efficacy. The antitumor activity may result from MFG-E8 down-regulation decreases tumor cell resistance to chemotherapy, perhaps related to a more powerful inhibition of tumor angiogenesis. Our study also found the enhanced induction of apoptosis in the tumor tissue, as well as a decrease of Treg cells in TILS from B16 mice. Although the mechanism for the interaction between MFG-E8 down-regulation and Dox needs further investigation, this combination of MFG-E8 RNAi and chemotherapy might be considered a new strategy for tumor treatment.

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Conflict of interest No potential conflicts of interest were disclosed.

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