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Dehydroxymethylepoxyquinomicin, a Novel Nuclear Factor-κB Inhibitor, Enhances Antitumor Activity of Taxanes in Anaplastic Thyroid Cancer Cells

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Nuclear factor κB (NF-κB), as an antiapoptotic factor, crucially affects the outcomes of cancer treatments, being one of the major culprits of resistance to chemotherapy. In this study, we investigated whether dehydroxymethylepoxyquinomicin (DHMEQ), a novel NF-κB inhibitor, can enhance antitumor activities of taxanes in anaplastic thyroid cancer (ATC) cells. Taxanes induced NF-κB activation in ATC cells, which could compromise the therapeutic effect of the drugs. However, DHMEQ, by inhibiting the nuclear translocation of NF-κB, completely suppressed the DNA binding capacities of NF-κB and lowered the levels of nuclear NF-κB protein. Compared with single treatment (either taxane or DHMEQ), the combined treatment strongly potentiated apoptosis, confirmed by cell survival assay; Western blotting for poly (ADP-ribose) polymerase, caspase 3, X-linked inhibitor of apoptosis, and survivin; and flow cytometry for annexin V. Furthermore, we also demonstrate for the first time that the combined treatment showed significantly greater inhibitory effect on tumor growth in a nude mice xenograft model. These findings suggest that taxanes are able to induce NF-κB activation in ATC cells, which could attenuate antitumor activities of the drugs, but inhibition of NF-κB by DHMEQ creates a chemosensitive environment and greatly enhances apoptosis in taxanes-treated ATC cells *in vitro* and *in vivo*. Thus, DHMEQ may emerge as an attractive therapeutic strategy to enhance the response to taxanes in ATCs. (*Endocrinology* 149: 5357–5365, 2008)

NUCLEAR FACTOR κB (NF-κB), named because it was first found to be a nucleoprotein able to bind to the enhancer region of the Igκ light chain gene, controls the expression of numerous gene products that play crucial roles in cell survival, angiogenesis, and carcinogenesis. In normal cells, NF-κB is strictly regulated, whereas in cancer cells, it is often constitutively activated to a high level (1). More importantly, NF-κB activation in cancer cells has been proven in many studies to be one of the major culprits of resistance to chemotherapy (2, 3). NF-κB is typically a heterodimeric complex composed of Rel family proteins p50 and p65. It usually resides in the cytoplasm in an inactive form due to its association with its inhibitor IκB. A number of extracellular signals can lead to NF-κB activation through the phosphorylation and degradation of IκB. Then liberated NF-κB translocates to the nucleus, binds to specific promoters, and regulates target gene expression.

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Abbreviations: ATC, Anaplastic thyroid cancer; DHMEQ, dehydroxymethylepoxyquinomicin; DMSO, dimethylsulfoxide; FITC, fluorescein isothiocyanate; IκB, inhibitor of NF-κB; NF-κB, nuclear factor-κB; PARP, poly (ADP-ribose) polymerase; TBST, Tris-buffered saline/0.1% Tween 20; WST, water-soluble tetrazolium salt; XIAP, X-linked inhibitor of apoptosis.

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Taxanes (including docetaxel and paclitaxel) break the equilibrium of microtubule polymerization by preventing tubulin depolymerization during mitosis, thus impairing cell proliferation in tumors. Taxanes have been used for several malignant tumors in clinics. Because no effective systemic treatment has been established for anaplastic thyroid cancer (ATC), taxanes could be a promising candidate. In fact, paclitaxel has been confirmed to be effective in ATC cells *in vitro* (4). However, it has been reported that taxanes also induce NF-κB activation in different types of malignant cells (5–10). Along with other reasons of intrinsic or acquired chemotherapy resistance to taxanes, such as β-tubulin mutations, different β-tubulin isotypes or multidrug resistance gene expression, this taxanes-induced NF-κB activation also attenuates the antitumor effect of the drugs and contributes to chemotherapy resistance (11).

NF-κB inhibitors have been considered as an appealing and target-oriented approach to deal with chemoresistant issue. A number of NF-κB inhibitors have been reported to be effective. Most of them, including curcumin (5, 12), genistein (13–15), parthenolide (16–18), BAY 11-7085 (9), and PS-1145 (6), act as IκB kinase (IKK) inhibitors. Dehydroxymethylepoxyquinomicin (DHMEQ), a NF-κB inhibitor designed from the structure of an antibiotic epoxyquinomicin C, inhibits NF-κB nuclear translocation (19, 20) as well as SN50 (21). It has been shown that DHMEQ is nontoxic and

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effective for ATC cells both *in vitro* and *in vivo* (22). As pointed out, DHMEQ inhibits NF-κB activity and up-regulates proapoptotic signaling in ATC cells, whereas normal thyroid epithelium cells are relatively resistant to the drug (22).

To our knowledge, there has been no study investigating the effect of docetaxel on ATC cells using an animal model. Docetaxel has been generally reported to be more effective and also less toxic than paclitaxel. Here we demonstrate that docetaxel is effective for ATC cells *in vitro* and *in vivo*. Furthermore, in a combined regimen, DHMEQ could greatly optimize the therapeutic effects of taxanes in ATC cells.

Materials and Methods

Reagents

Racemic DHMEQ was dissolved in dimethylsulfoxide (DMSO) (Wako Chemicals, Osaka, Japan) at a stock concentration of 10 mg/ml and then stored at −20 C. Docetaxel and paclitaxel (Wako Chemicals) were dissolved in DMSO at a stock concentration of 1 mM for *in vitro* experiments, and for *in vivo* experiments, docetaxel was dissolved at 20 mg/ml. SN50 was purchased from Calbiochem (San Diego, CA). Antibodies were obtained from the following sources: anti-p50 polyclonal, p65 polyclonal, survivin polyclonal, and β-actin monoclonal from Santa Cruz Biotechnology (Santa Cruz, CA) and anti-IκBα polyclonal, X-linked inhibitor of apoptosis (XIAP) polyclonal, poly (ADP-ribose) polymerase (PARP) polyclonal, cleaved caspase 3 polyclonal, antirabbit IgG, and antimouse IgG horseradish peroxidase-conjugated secondary antibodies from Cell Signaling Technology (Beverly, MA).

Cell culture

Human ATC cell lines ARO and FRO were initially provided by Dr. James A. Fagin (University of Cincinnati, College of Medicine, Cincinnati, OH). KTC-2 cells were from Dr. Kurebayashi (Kawasaki Medical School, Kurashiki, Japan) (23). All cells were grown in RPMI 1640 (Sigma Chemical Co., St. Louis, MO) supplemented with 5% fetal bovine serum and 1% (wt/vol) penicillin/streptomycin (Sigma) in a 5% CO_2 humidified atmosphere at 37 C.

Cell survival assay

Cell suspensions (100 μl, 3000 cells per well) were added to each well of a 96-well plate and incubated for 24 h before treatment. Solutions containing various concentrations of taxanes and/or DHMEQ or SN50

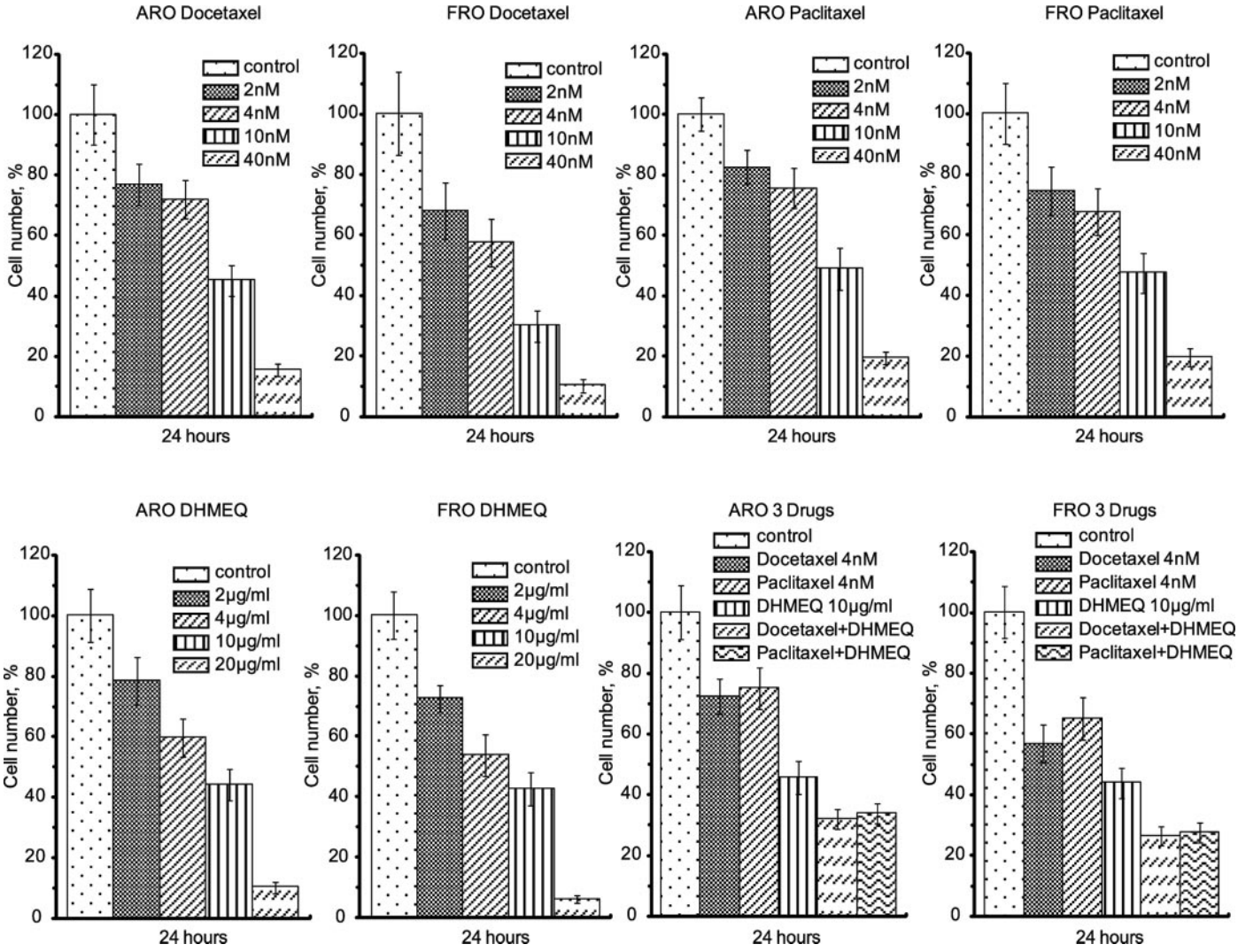

FIG. 1. Cytotoxic effect of taxanes and/or DHMEQ on ATC cells. Viabilities of FRO and ARO cells exposed to indicated concentrations of docetaxel, paclitaxel, and/or DHMEQ for 24 h were determined by WST assay as described in *Materials and Methods*. *Bars* represent the mean ± SD of four wells. Similar results were obtained in at least three independent experiments.

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were added to each well in 11 μl medium, with four wells used for each concentration. In the control wells, DMSO was added, and the final concentration of DMSO in any well did not exceed 0.2%. After incubation, a water-soluble tetrazolium salt (WST)-based assay was implemented as follows: first, old medium was aspirated, then 50 μl fresh RPMI 1640 was added, and finally 5 μl CKK-8 solution (Dojindo, Osaka, Japan) was added to each well and incubated for 60 min at 37 C. ODs were measured at 450 nm in a microplate reader ImmunoMini NJ-2300 (System Instruments, Tokyo, Japan).

Preparation of cell extracts

For total cell extracts, adherent cells were washed twice with ice-cold PBS, scraped with a rubber policeman, collected in 1 ml PBS, and centrifuged for 3 min at 1000 rpm at 4 C. The pellet was then resuspended in 100 μl lysis buffer [20 mM Tris-HCl (pH 7.5), 1 mM EDTA, 150 mM NaCl, 0.5% Triton X-100, 50 mM NaF, 10 mM sodium pyrophosphate, 1 mM sodium orthovanadate, and 2 mM phenylmethylsulfonyl fluoride) containing protease inhibitor cocktail (Roche Diagnostics, Basel, Switzerland). After incubation for 20 min on ice, the lysate was centrifuged for 15 min at 14,000 rpm. The supernatant was stored at −80 C until use. Nuclear extracts were prepared according to the method of Andrews and Faller (24) with some modifications. In brief, attached cells were harvested, washed with ice-cold PBS, suspended in 400 μl buffer A [10 mM HEPES (pH 7.9), 10 mM KCl, 1.5 mM $MgCl_2$, 0.5 mM dithiothreitol, 0.5 mM phenylmethylsulfonyl fluoride, and 0.1% Nonidet P-40] and incubated on ice for 20 min. Nuclei were pelleted by centrifugation for 5 min at 14,000 rpm, resuspended in 40 μl buffer C [20 mM HEPES (pH 7.9), 420 mM NaCl, 1.5 mM $MgCl_2$, 1 mM dithiothreitol, 0.2 mM EDTA, 0.5 mM phenylmethylsulfonyl fluoride, and 20% glycerol], incubated on ice for 20 min, and centrifuged for 15 min at 14,000 rpm at 4 C. The supernatant was also stored at −80 C. Protein concentrations were determined with a bicinchoninic acid assay reagent kit (Sigma).

DNA-binding assay

The multiwell colorimetric assay for active NF-κB was performed as described previously (25, 26). Briefly, equal amounts of nuclear extracts were incubated in a 96-well plate coated with immobilized oligonucleotide containing a NF-κB consensus binding site. NF-κB binding to the target oligonucleotide was detected with primary antibody specific to p65 subunit and horseradish peroxidase-conjugated secondary antibody. For quantification of activity, ODs were measured at 450 nm using a microplate reader ImmunoMini NJ-2300.

Western blotting

Equal amounts of protein were separated by SDS-PAGE in 10 or 15% polyacrylamide gels. Proteins were transferred onto nitrocellulose membranes (Pall Corp., Ann Arbor, MI) by semidry blotting. Membranes were blocked with Tris-buffered saline/0.1% Tween 20 (TBST) containing 1% nonfat dry milk for 60 min at room temperature. After washing three times with TBST, membranes were incubated with appropriately diluted primary antibodies at 4 C overnight. After washing three times with TBST, the blots were incubated with horseradish peroxidase-conjugated species-specific secondary antibody for 1 h at room temperature and then again washed three times. Then the complexes were visualized in an LAS-3000 imaging system (FUJIFILM, Tokyo, Japan) by using the enhanced chemiluminescence reagents (Nacalai Tesque, Kyoto, Japan).

Flow cytometry analysis with the annexin V/propidium iodide staining

Adherent cells were harvested by trypsinization, and 4×10^5 cells were double stained with fluorescein isothiocyanate (FITC)-conjugated annexin V and propidium iodide for 15 min at room temperature in a Ca^{2+}-enriched binding buffer (Apoptosis Detection Kit; Wako Chemicals) and then analyzed on a FACS Vantage SE System flow cytometer (BD Biosciences, San Jose, CA). FITC and propidium iodide emissions were detected in FL-1 and FL-3 channels, respectively. Analysis was done with CellQuest software (BD Biosciences).

In vivo xenograft model

All procedures involving animal experiments and their care in this study were conducted in accordance with the principles and procedures outlined in the Guide for the Care and Use of Laboratory Animals at

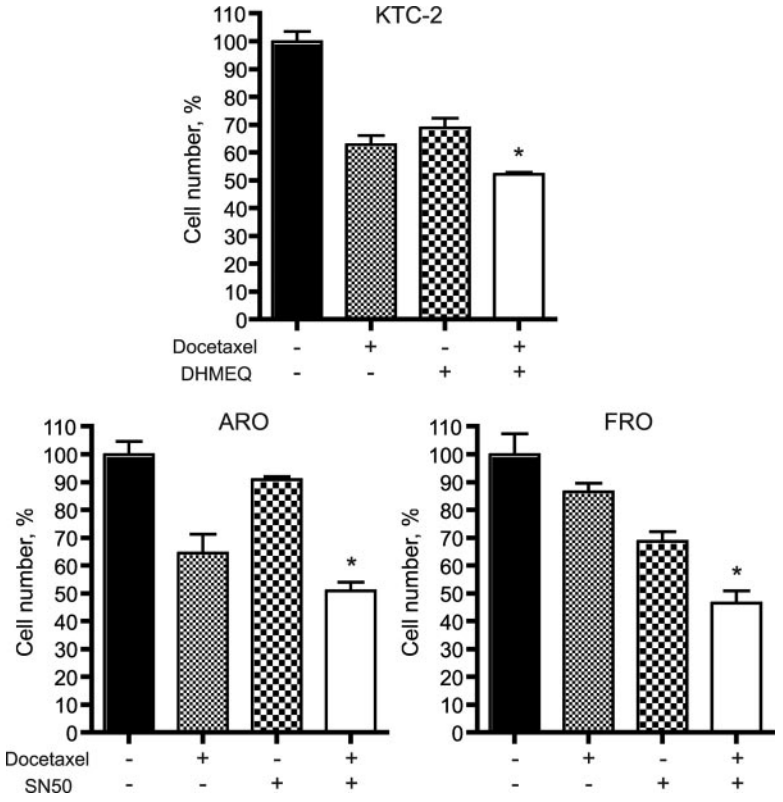

FIG. 2. Combined effects were also observed in cells with wild-type p53 or in cells treated with SN50. Viability of indicated cells exposed to 4 nM docetaxel and/or 10 μg/ml DHMEQ or 50 μg/ml SN50 for 24 h were determined by WST assay as described in *Materials and Methods*. *Bars* represent the mean and SD of four wells. Similar results were obtained in at least three independent experiments. *, $P < 0.05$ *vs.* other groups.

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Nagasaki University. FRO cells (5×10^6) resuspended in RPMI 1640 were injected sc into both flanks of 6-wk-old male BALB/c *nu/nu* mice (CLEA Japan, Tokyo, Japan), six animals per group. Then they were randomly assigned into four groups. The tumor sizes were measured each alternate day with calipers, and tumor volumes were calculated according to the formula $a^2 \times b \times 0.4$, where a is the smallest tumor diameter and b is the diameter perpendicular to a. DHMEQ, diluted in PBS/DMSO (ratio 1:1), was injected ip at a dose of 6 mg/kg·d for 14 d, beginning from d 5 after tumor implantation. Docetaxel, diluted in the same way, was injected ip at a dose of 5 mg/kg on d 5 and 12. Combined treated mice were given both drugs. Control group mice received vehicle injections only. For two more weeks, tumor size was monitored, and body weight, feeding behavior, and motor activity of each animal were used as indicators of general health.

Statistical analysis

All data are expressed as the mean ± SD. Differences between groups were examined for statistical significance with one-way ANOVA followed by Tukey's post test. A P value not exceeding 0.05 was considered statistically significant.

Results

Cytotoxic effect of taxanes and/or DHMEQ on ATC cells

Because both paclitaxel and DHMEQ have been reported to have cytotoxic effects on ATC cells, whereas normal thyrocytes exhibit significantly lower sensitivity to them (4, 22), in the current experiment, we first determined the proper concentrations of the drugs to use. FRO and ARO cells were treated with different concentrations of docetaxel, paclitaxel, and/or DHMEQ for 24 h, and then a cell survival assay was done. Survival rates of ATC cells showed an inverse relationship to the dosage of any drug (Fig. 1). For *in vitro* experiments, concentrations of taxanes and DHMEQ were determined as 4 nM and 10 μg/ml, respectively. As shown in Fig. 1, the combined treatment strongly enhanced the growth-inhibitory effect compared with single treatment (Fig. 1).

Combined effects were also observed in cells with wild-type p53 or in cells treated with SN50

Because both ARO and FRO cells harbor a *TP53* mutation, we used KTC-2 cells also derived from ATC but having no *TP53* mutation (our unpublished data) to check the effect of the mutational status of *TP53*. As shown in Fig. 2, the combined treatment also showed significant enhancement compared with single treatment. We next used SN50, another NF-κB inhibitor, to confirm the effect of the combination. Although SN50 was shown to inhibit nuclear translocation of NF-κB, nuclear factor of activated T cells and activator protein 1 at a high concentration (210 μg/ml) (27), lower doses of SN50 (37.5 μg/ml) selectively inhibited NF-κB translocation (28). In both ARO and FRO cells, the combined treatment (docetaxel and SN50) similarly enhanced growth inhibition (Fig. 2), suggesting that inhibition of NF-κB activation induces a taxane-sensitive environment in ATC cells. Note that the effect of SN50 was smaller than that of DHMEQ. This is probably due to prolonged c-Jun N-terminal kinase activation by DHMEQ, whereas SN50 did not activate c-Jun N-terminal kinase signaling. We have reported this in a previous paper (22).

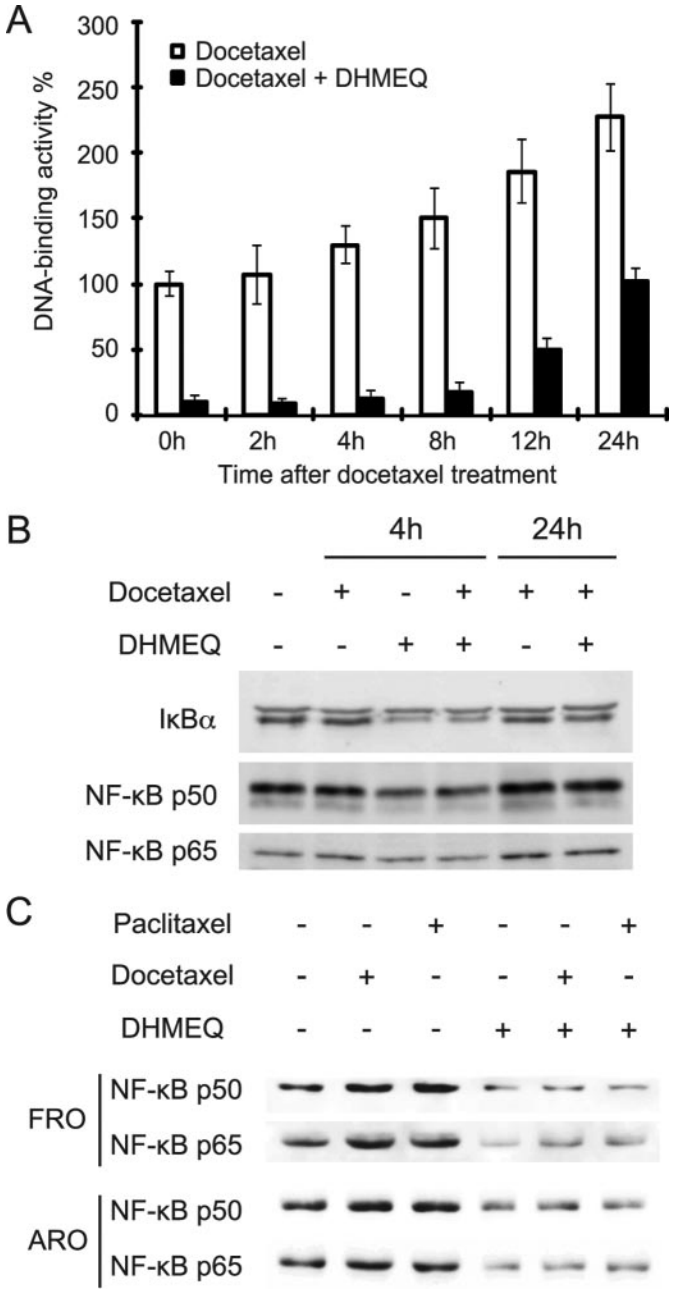

FIG. 3. Taxanes induce NF-κB activation, and DHMEQ inhibits the effect. A, DNA-binding assay. FRO cells were preincubated with or without 10 μg/ml DHMEQ for 1 h and then treated with 4 nM docetaxel for 24 h. Nuclear extracts were prepared, and DNA-binding assays were done as described in *Materials and Methods*. *Bars* represent the mean ± SD of three wells. Similar results were obtained in three independent experiments. B, Western blotting. FRO cells were treated with 4 nM docetaxel and/or 10 μg/ml DHMEQ for the indicated times, and then total cell lysates (for IκBα) or nuclear protein extracts (for p50 and p65) were examined using the indicated primary antibodies. Similar results were obtained in three independent experiments. C, Western blotting. The indicated cells were treated with 4 nM taxanes and/or 10 μg/ml DHMEQ for 4 h, and then nuclear protein extracts were examined for indicated subunit of NF-κB by Western blotting. Similar results were obtained in three independent experiments.

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Effects of taxanes and DHMEQ on NF-κB in ATC cells

To examine the effects of taxanes on NF-κB signaling in ATC cells, we performed a DNA-binding assay using nuclear extracts from drug-treated FRO cells, which have already been reported to possess a high level of constitutively active NF-κB (29). The cells were treated with docetaxel for 24 h, and during this time course, the binding activity of nuclear p65 was gradually increased (Fig. 3A). However, if the cells were pretreated with DHMEQ for 1 h and then exposed to docetaxel, the level of p65 was suppressed, especially during the first 8 h (Fig. 3A). We also checked IκBα expression after the treatment. Consistent with our previous data (22), DHMEQ treatment decreased IκBα protein level. NF-κB is known to bind the IκB promoter and activate its synthesis, and therefore the inhibition of NF-κB by DHMEQ probably suppressed *de novo* synthesis of IκBα. Presumably, for the same reason, IκBα expression after treatment with docetaxel was not dramatically reduced (Fig. 3B). On the other hand, nuclear p50 and p65 levels were clearly suppressed by DHMEQ treatment at 4 h but recovered at 24 h, mostly consistent with the result of DNA-binding assay (Fig. 3, A and B). The amount of nuclear p50 and p65 was slightly increased in the paclitaxel as well as docetaxel group but decreased in the DHMEQ and combination treatment groups in both ARO and FRO cells (Fig. 3C). These results confirmed not only the phenomenon of taxane-induced NF-κB activation but also that DHMEQ can inhibit the effect, just as DHMEQ has been reported to prevent unstimulated, TNF-α-induced or chemotherapeutic agent-induced accumulation of NF-κB subunits in cancer cell lines (20, 22, 30–32).

Both taxanes and DHMEQ can induce apoptosis

Because cleavages of caspase 3 (a key executioner of apoptosis) and PARP (a main cleavage target of caspase 3) are characteristic indices of apoptosis, we examined their changes by Western blotting. After treatment with taxanes or DHMEQ for 24 h, any agent induced cleavage of PARP (p89) and caspase 3 (p19 and p17) (Fig. 4, A and B), consistent with previous reports (22, 33–36). The cleaved PARP and caspase 3 levels were further increased by the combined treatment, suggesting that more ATC cells underwent apoptosis (Fig. 4, A and B). During the 24-h time course of the treatment with docetaxel, the level of cleaved PARP and caspase 3 was gradually and slowly increased up to 24 h (Fig. 4C). On the other hand, pretreatment with DHMEQ for 1 h caused both cleavages much earlier and even stronger (Fig. 4C).

Synergistic apoptosis detected by flow cytometry

To further confirm the effects of combined treatment on apoptosis, the cells were treated with the drugs for 16 h and then double stained with FITC-conjugated annexin V and

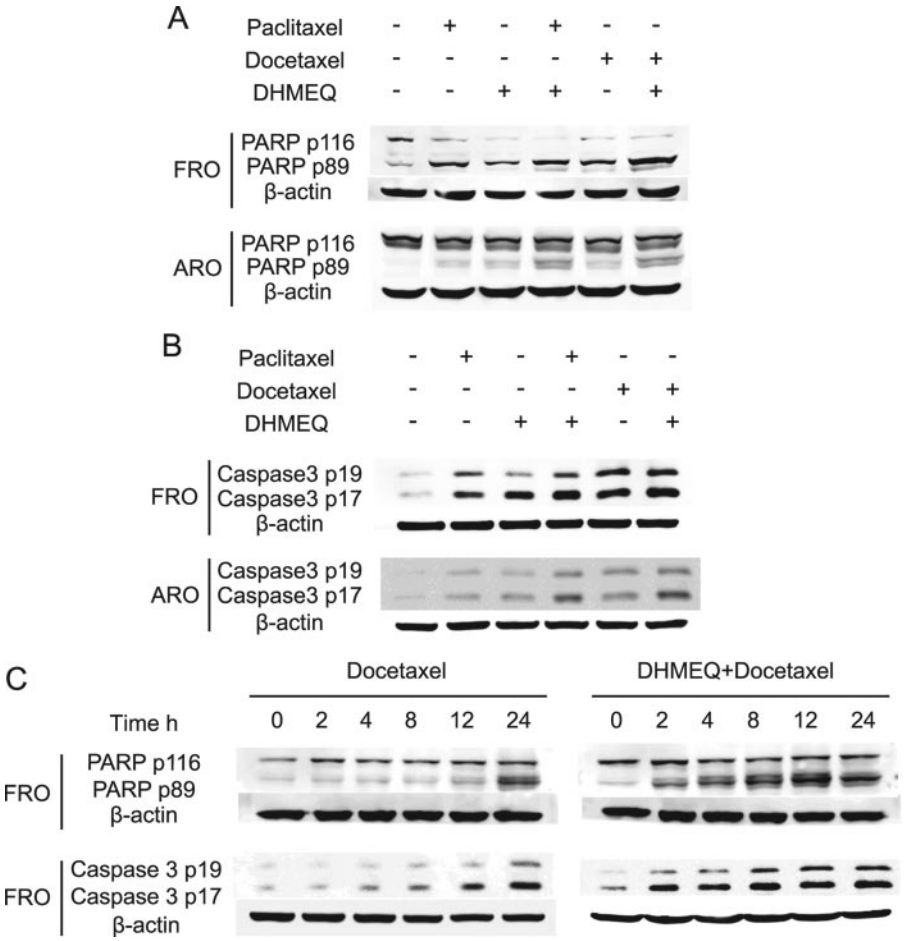

FIG. 4. Effect of taxanes and DHMEQ on PARP cleavage and caspase 3 activation. A and B, Indicated cells were treated with the indicated drugs for 24 h (4 nM taxanes and/or 10 μg/ml DHMEQ), and whole-cell lysates were examined by Western blotting for PARP (A) and caspase 3 (B). C, FRO cells were preincubated with or without 10 μg/ml DHMEQ for 1 h and then treated with 4 nM docetaxel for the indicated times. Whole-cell lysates were examined by Western blotting for PARP and caspase 3. A–C, β-Actin was used as a loading control. Similar results were obtained in at least three independent experiments.

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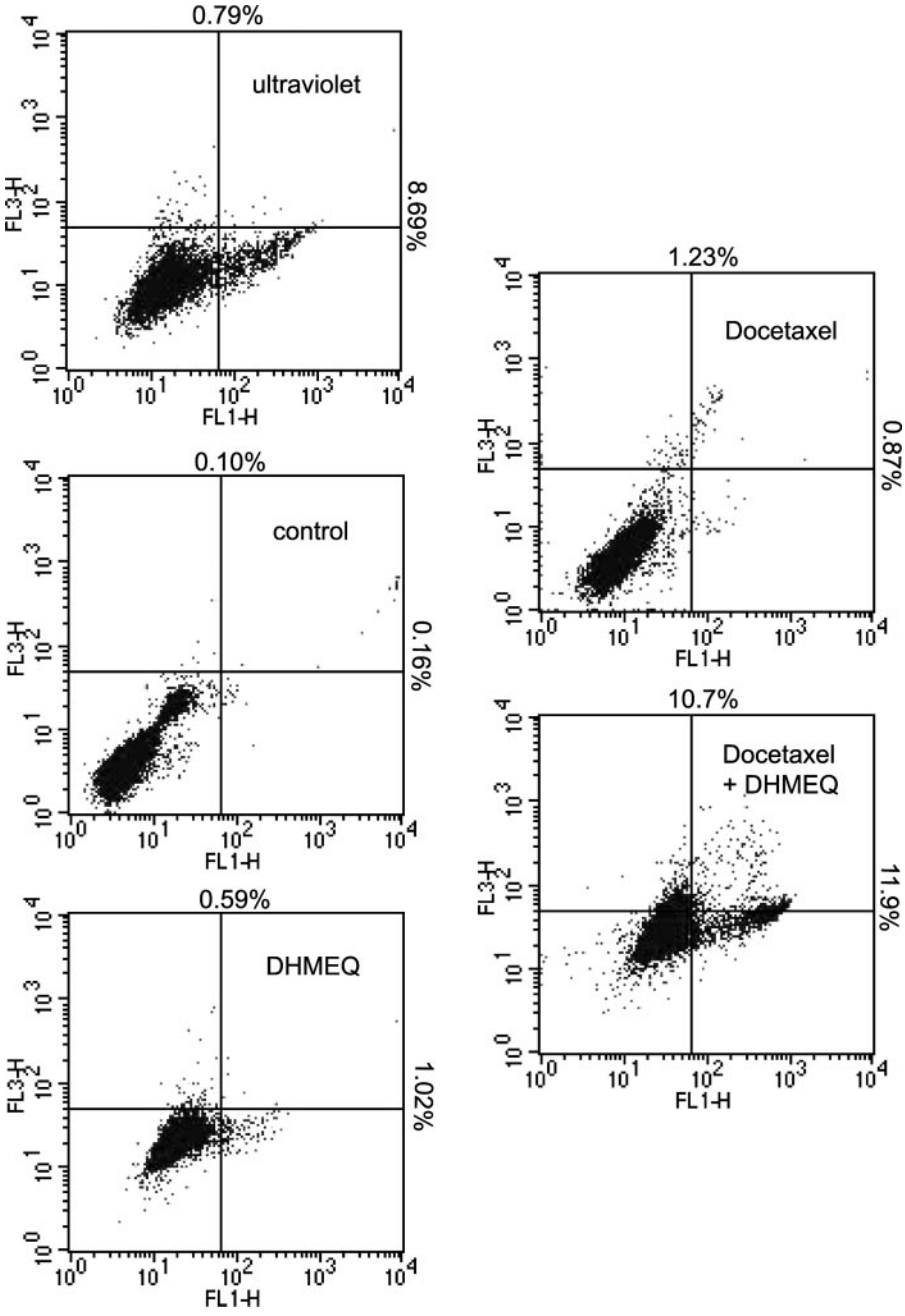

FIG. 5. Apoptotic changes in cells treated with drugs. Five groups of FRO cells with different treatments (4 nM docetaxel, 10 μg/ml DHMEQ, docetaxel plus DHMEQ, 10 J/m^2 UV, and control with DMSO only) for 16 h were harvested by trypsinization and subjected to annexin V/propidium iodide apoptosis detection assay using a FACS Vantage SE flow cytometer. FITC and propidium iodide emissions were detected in the FL-1 and FL-3 channels, respectively. The percentage of annexin V-positive cells (on the *right*) represents the sums of *upper right* and *lower right* quadrants, and the percentage of propidium iodide-positive cells (on the *top*) represents the sums of *top left* and *top right* quadrants. Similar results were obtained in two independent experiments.

propidium iodide. Although either drug slightly induced apoptosis, combined treatment synergistically increased apoptosis (Fig. 5).

Western blotting of antiapoptotic factors

XIAP and survivin, belonging to the human inhibitors of apoptosis (IAP) family, are target genes regulated by NF-κB, and they are overexpressed in many cancers (1, 37). We tested whether taxanes and/or DHMEQ modulate the expression of these antiapoptotic gene products. Besides high basal levels of XIAP and survivin in ATC cell lines, taxanes further increased their levels (Fig. 6). However, when treated with DHMEQ, their levels were markedly reduced to even less than basal level (Fig. 6). These data indicate that both constitutive high levels and taxanes-induced XIAP and survivin expressions can be successfully suppressed by DHMEQ, consistent with our previous reports (4, 22) and papers from others (31, 38).

In vivo effects of the combined treatment with taxanes and DHMEQ

To explore the effects of the combined treatment *in vivo*, we used an animal xenograft model inoculated with FRO cells. As shown in Fig. 7, treatment with either drug was able to delay tumor growth, but the effect of the combined treatment with DHMEQ and docetaxel was far greater, with three locations (in two mice) remarkably having no apparent tumor.

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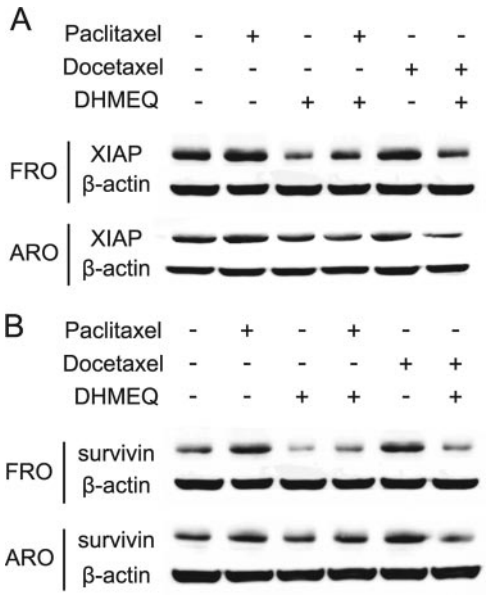

FIG. 6. Effect of taxanes and DHMEQ on antiapoptotic factors. Indicated cells were treated with 4 nM taxanes and/or 10 μg/ml DHMEQ for 24 h, and whole-cell lysates were examined by Western blotting for XIAP (A) and survivin (B). β-Actin was used as a loading control. Similar results were obtained in at least three independent experiments.

During the course of the therapy, no changes in behavior and body weight were observed.

Discussion

Although taxanes have been shown to possess powerful cell-killing abilities in a variety of cancer cells including ATC (4), they have also been reported to induce NF-κB activation in several types of malignant cells, including breast (5, 17, 18), ovarian (9, 12), prostate (13, 16), pancreatic (8, 14), gastric (7), and lung (39) cancers. The present data showed that taxanes induced NF-κB activation in ATC cells as well. NF-κB activation mediates survival signals that counteract apoptosis and could greatly compromise the therapeutic effect of taxanes. Adjuvant attempts to inhibit NF-κB and increase the therapeutic efficacies of taxanes have been reported for all of the above mentioned cancer cell lines. However, there have been no reports regarding a NF-κB inhibitor in an adjuvant setting with taxanes for ATC cells. We demonstrated in the current study that although taxanes induced NF-κB activation in ATC cells, DHMEQ could effectively inhibit the translocation of NF-κB, suppress antiapoptotic factors, and greatly enhance apoptosis. We also showed for the first time that a combination of DHMEQ and docetaxel was much more effective in the inhibition of tumor growth than monotreatment in nude mice xenograft models.

As a selective NF-κB inhibitor, DHMEQ is able to decrease transcription of many antiapoptotic genes and then lower the threshold of triggering apoptosis. Therefore, when used in combination with subtoxic concentrations of chemotherapeutic drugs, DHMEQ could create a chemosensitizing environment in various cancers. This is especially beneficial for tumors with intrinsic or acquired drug resistance (3). Consistent with our findings, several studies of applying DHMEQ in the armamentarium of anticancer therapeutics have been carried out, and synergistic effects were shown (32, 38, 40–42). Ruan *et al.* (40) used low concentrations of DHMEQ (1.0 or 5.0 μg/ml) to enhance the sensitivity of two head and neck squamous cell carcinoma cell lines to cisplatin, and synergistic cytotoxic effects were observed. Poma *et al.* (32) used DHMEQ as a sensitizing agent in hepatic cancer. DHMEQ (5.0 μg/ml) also exhibited synergy with cisplatin, decreasing the levels of prosurvival genes and IL-6 production. Horie *et al.* (41) used DHMEQ (5.0 μg/ml) in combination with fludarabine to treat chronic lymphocytic leukemia. DHMEQ abrogated both constitutive and induced NF-κB activities and enhanced fludarabine-induced apopto-

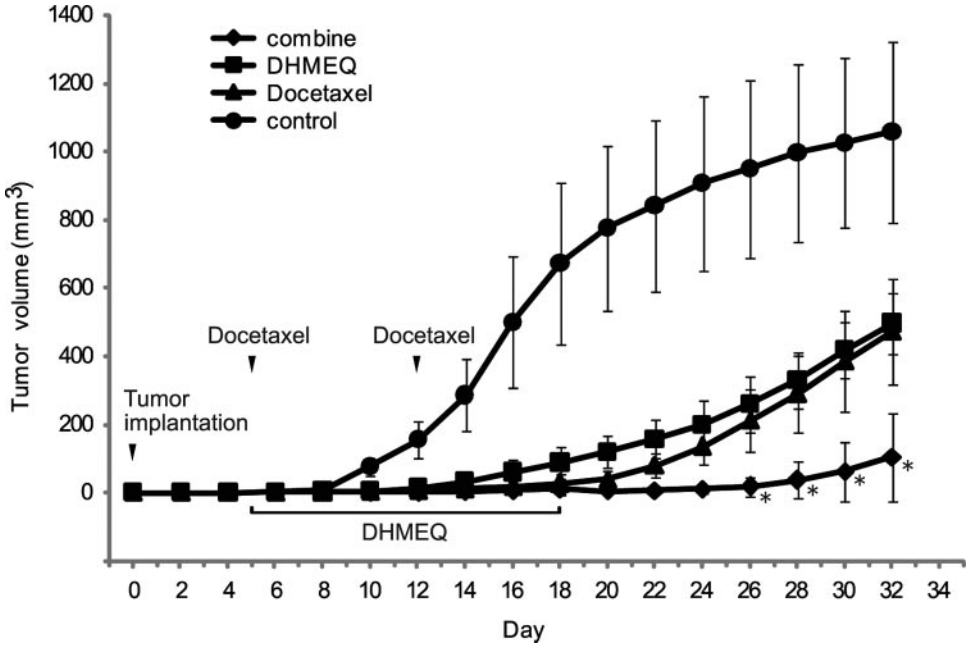

FIG. 7. Effect of docetaxel and DHMEQ in FRO tumor xenograft model. FRO cells (5×10^6) were implanted as described in *Materials and Methods*. DHMEQ was injected ip at a dose of 6 mg/kg·d for 14 d, beginning on d 5 after tumor implantation. Docetaxel was injected ip at a dose of 5 mg/kg on d 5 and 12. Combined treatment mice were given both drugs. Control group mice received vehicle injections only. In control groups, tumors did not appear at three locations (in two mice) until d 32. Data are presented as the mean ± SD of 12 tumors (in six mice). *, $P < 0.05$ *vs.* any other group.

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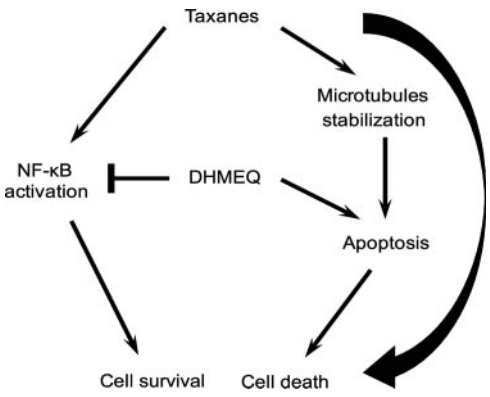

FIG. 8. Proposed mechanism of combined treatment. In ATC cells, taxanes bind to microtubules, impair mitosis, and induce apoptosis, yet at the same time, they also induce NF-κB activation, leading to dampening the effect of taxanes. DHMEQ blocks taxanes-induced NF-κB activation, and together with its own cell-killing effect, cancer cell apoptosis is greatly enhanced.

sis. Besides, the combination of DHMEQ (10 μg/ml) and interferon-γ has been reported to synergistically inhibit renal cancer cells' proliferation (38). Very recently, Jazirehi *et al.* (42) tested combinations of DHMEQ with several chemotherapeutic drugs in non-Hodgkin's lymphoma. The wild-type cells were pretreated with DHMEQ (10 μg/ml), whereas the rituximab-resistant clones were pretreated with a higher concentration of DHMEQ (20 μg/ml). All cells were then incubated with subtoxic concentrations of paclitaxel, adriamycin, cisplatin, vincristine, and etoposide and then subjected to DNA fragmentation assay to measure apoptosis. DHMEQ chemosensitized both types of cells, suggesting that DHMEQ successfully reversed the cells from chemoresistant to chemosensitive.

Based on our and others' findings, we are led to propose a mechanistic scheme, as shown in Fig. 8, elucidating the enhanced induction of apoptosis by DHMEQ and taxanes. In ATC cells, dual effects of taxanes exist: they bind to microtubules, impair mitosis, and induce apoptosis. However, at the same time, they can also induce NF-κB activation, leading to cell survival. DHMEQ can block the nuclear translocation of NF-κB and promote apoptosis. By this mechanism, DHMEQ can presumably modulate the balance between pro- and antiapoptotic signals and enhance taxanes-induced apoptosis synergistically.

Collectively, we presented *in vitro* and for the first time *in vivo* evidence showing that DHMEQ could abrogate NF-κB activation induced by the chemotherapeutic agent taxanes and create a more favorable proapoptotic environment, leading to enhancement of the killing effect in the combined regimen. NF-κB inhibitor SN50 also showed enhanced effects in combination with radiotherapy, perhaps through the same mechanism (29). Thus, DHMEQ, as an effective NF-κB inhibitor, could be applicable in several combination regimens.

In clinical practices, combined treatment is commonly considered to achieve better therapeutic outcome. However, sometimes it may cause adverse side effects and systemic toxicities, which can be devastating for advanced-stage cancer patients. DHMEQ, being an antibiotic derivative, has very few side effects as shown in many *in vivo* experiments (30, 33, 34, 43–47), and we also reported that normal thyroid epithelial cells are resistant to DHMEQ (22). Thus, as the combination decreases the dosage of taxanes, it can circumvent the unwanted toxicity of taxanes, and above all therapeutic effects increase. It would be promising that the molecular target-oriented coadministration of DHMEQ and taxanes will emerge as an attractive therapeutic strategy for ATC patients.

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