First Evidence That γ-Tocotrienol Inhibits the Growth of Human Gastric Cancer and Chemosensitizes It to Capecitabine in a Xenograft Mouse Model through the Modulation of NF-κB Pathway

Kanjoomana A. Manu1, Muthu K. Shanmugam1, Lalitha Ramachandran1, Feng Li1, Chee Wai Fong3, Alan Prem Kumar1,2,6, Patrick Tan2,4,5, and Gautam Sethi1,2

Abstract

Purpose: Because of poor prognosis and development of resistance against chemotherapeutic drugs, the existing treatment modalities for gastric cancer are ineffective. Hence, novel agents that are safe and effective are urgently needed. Whether γ-tocotrienol can sensitize gastric cancer to capecitabine in vitro and in a xenograft mouse model was investigated.

Experimental Design: The effect of γ-tocotrienol on proliferation of gastric cancer cell lines was examined by mitochondrial dye uptake assay, apoptosis by esterase staining, NF-κB activation by DNA-binding assay, and gene expression by Western blotting. The effect of γ-tocotrienol on the growth and chemosensitization was also examined in subcutaneously implanted tumors in nude mice.

Results: γ-Tocotrienol inhibited the proliferation of various gastric cancer cell lines, potentiated the apoptotic effects of capecitabine, inhibited the constitutive activation of NF-κB, and suppressed the NF-κB–regulated expression of COX-2, cyclin D1, Bcl-2, CXCR4, VEGF, and matrix metalloproteinase-9 (MMP-9). In a xenograft model of human gastric cancer in nude mice, we found that administration of γ-tocotrienol alone (1 mg/kg body weight, intraperitoneally 3 times/wk) significantly suppressed the growth of the tumor and this effect was further enhanced by capecitabine. Both the markers of proliferation index Ki-67 and for microvessel density CD31 were downregulated in tumor tissue by the combination of capecitabine and γ-tocotrienol. As compared with vehicle control, γ-tocotrienol also suppressed the NF-κB activation and the expression of cyclin D1, COX-2, intercellular adhesion molecule-1 (ICAM-1), MMP-9, survivin, Bcl-xL, and XIAP.

Conclusions: Overall our results show that γ-tocotrienol can potentiate the effects of capecitabine through suppression of NF-κB–regulated markers of proliferation, invasion, angiogenesis, and metastasis.

Clin Cancer Res; 18(8); 2220–9. ©2012 AACR.

Introduction

Gastric cancer remains one of the most common malignancies and the second leading cause of cancer mortality accounting for more than 600,000 deaths annually worldwide (1, 2). Chemotherapy constitutes an important treatment regimen for gastric cancers besides surgical resection (3). Unfortunately, only few patients experience complete pathologic response to chemotherapeutic drugs like capecitabine, mainly because of their resistance to chemotherapy (4). Hence, novel approaches to enhance the effects of chemotherapeutic drugs and reduce their resistance are imperative.

Various lines of evidence suggest that the activation of master transcription factor NF-κB and its regulated gene products play a pivotal role in growth, metastasis, and chemoresistance of gastric cancer. First, NF-κB is constitutively activated in human gastric cancer tissue and is associated with tumor progression (5). Second, it promotes gastric cancer growth by inhibiting apoptosis (6). Third, NF-κB mediates the induction of mitogenic gene products such as cyclin D1, which is overexpressed in human gastric cancer tissue and is inversely correlated with poor prognosis.
Translational Relevance

Despite advances in earlier detection and therapy for gastric cancer, it still remains the second leading cause of cancer death worldwide, killing more than 600,000 people annually worldwide. Existing drugs lack efficacy and yet are highly toxic. For example, although capecitabine is used routinely in the treatment of gastric cancer, development of resistance to this treatment in the patients is one of the major problems. Thus agents which can overcome the resistance and can enhance the effect of capecitabine are urgently needed. Through in vitro and in vivo experiments, we show for the first time that γ-tocotrienol, a vitamin E analogue, is one such agent that can reduce the resistance and can potentiate the effect of capecitabine against gastric cancer. Because γ-tocotrienol is already in clinical trials, the present study may form the basis of novel therapeutic options for the treatment of patients with gastric cancer.

and poor survival (7). Fourth, NF-kB plays an important role in regulating the CXCR4 motif receptor 4 (CXCR4; ref. 8) and COX-2 (9) that are associated with gastric cancer metastasis. Finally, it is well established that Helicobacter pylori, a well-known risk factor for gastric carcinoma, is a potent activator of NF-kB in gastric epithelial cells (10). Moreover, NF-kB pathway is also responsible for the increased generation of several cell adhesion molecules including intercellular adhesion molecule-1 (ICAM-1), whose expression is significantly correlated with an increase in H. pylori–induced gastritis (11). Together, these findings implicate the involvement of NF-kB pathway in gastric cancer and thus the agents that can modulate NF-kB and NF-kB–regulated gene products have enormous potential for the treatment of gastric cancer.

One such agent derived from natural sources that can have a great potential for gastric cancer prevention and treatment is vitamin E constituent, γ-tocotrienol derived from palm oil and rice bran. Vitamin E family of compounds primarily consists of 4 tocotrienols and 4 tocopherols (12). However, while tocopherols had been intensively studied for their health benefits, many novel benefits of tocotrienols are only beginning to be brought to light by research in the last decade (13, 14). For instance, γ-tocotrienol has been reported to suppress the proliferation of a wide variety of tumor cells (15), including gastric (16–19), hepatocellular carcinoma (20), melanoma (21), breast (22), colorectal (23), and prostate (24). In vivo mice studies have shown that γ-tocotrienol can suppress the growth of breast tumor (25), prostate (26), lung cancer and melanoma (27) and also inhibit the growth of liver and pancreatic cancer either alone or in combination with chemotherapeutic drugs and radiation (28, 29). How γ-tocotrienol mediates its anticancer effects is not completely understood, but the roles of various signaling cascades/kinases/transcription factors such as mitogen-activated protein kinases (17), phosphoinositide 3-kinase (PI3K)/Akt (30), NF-kB (13), STAT3 (20), telomerase (31), PPAR-γ (32), hypoxia-inducible factor-1α (33), β-catenin (23), EGF (24), and inhibitor of differentiation family proteins (34) have been implicated.

Although γ-tocotrienol has been found to suppress proliferation, to inhibit invasion/migration, and induce apoptosis in human gastric cancer SGC-7901 cells (28–31), but its potential to act as a chemosensitizing agent in gastric cancer cell lines and xenograft models has never been explored before (16–19). Thus, in the present study, we investigated whether γ-tocotrienol could sensitize human gastric cancer to capecitabine in vitro and in a xenograft mouse model. Our observations indicate for the first time that γ-tocotrienol can inhibit the proliferation of various gastric cancer cells, enhanced capecitabine-induced apoptosis, and potentiated the antitumor activity of capecitabine in human xenograft gastric cancer model through the modulation of NF-kB and NF-kB–regulated gene products.

Materials and Methods

Reagents

γ-Tocotrienol with purity more than 97% was obtained from Davos Life Science. MTT, Tris base, glycine, NaCl, SDS, bovine serum albumin (BSA), β-actin antibody, and corn oil were purchased from Sigma-Aldrich. γ-Tocotrienol was dissolved in dimethylsulfoxide as a 10 mmol/L stock solution and stored at 4°C for in vitro and in vivo experiments, respectively. Further dilution was done in cell culture medium. RPMI-1640 media, FBS, 0.4% trypan blue vital stain, and antibiotic–antimycotic mixture were obtained from Invitrogen. Antibodies against p65, matrix metalloproteinase-9 (MMP-9), Bcl-2, Bcl-xl, COX-2, ICAM-1, cyclin D1, survivin, Mcl-1, VEGF, and XIAP were obtained from Santa Cruz Biotechnology. CXCR4 antibody was obtained from Abcam. CD31 antibody was purchased from Cell Signaling Technology. Ki-67 antibody was purchased from BD Pharmingen, Inc. Goat anti-rabbit horseradish peroxidase (HRP) conjugate and goat anti-mouse HRP were purchased from Duheng International Trading Company Ltd. and dissolved in sterile PBS on the day of use.

Cell lines

The gastric cancer cell lines SNU-5 and SNU-16 were obtained from the American Type Culture Collection. MKN45 cells were obtained from JCRB (Japanese Collection of Research Bioresources), Japan. All the gastric cancer cell lines were cultured in RPMI-1640 media supplemented with 10% FBS, 100 units/mL penicillin, and 100 μg/mL streptomycin.

Western blotting

For detection of various proteins, gastric cancer cells (2 × 10⁶ per mL) were treated with γ-tocotrienol for different time intervals. The cells were then washed and extracted by incubation for 30 minutes on ice in 0.05 mL buffer containing 20 mmol/L HEPES, pH 7.4, 2 mmol/L EDTA,
250 mmol/L NaCl, 0.1% NP-40, 2 μg/ml leupeptin, 2 μg/ml aprotinin, 1 mmol/L phenylmethylsulfonylfluoride (PMSF), 0.5 μg/ml benzamidine, 1 mmol/L DTT, and 1 mmol/L sodium vanadate. The lysate was centrifuged, and the supernatant was collected. Whole-cell extract protein (30 μg) was resolved on 12% SDS-PAGE, electroblotted onto a nitrocellulose membrane, blocked with antibodies against survivin, Bcl-2, Bcl-xl, cyclin D1, VEGF, procaspase-3, and PARP and then detected by chemiluminescence (ECL; GE Healthcare).

Cell proliferation MTT assay
The effect of γ-tocotrienol on cell proliferation was determined by the MTT uptake method as described previously (20). The cells (5,000 per well) were incubated with γ-tocotrienol in triplicate in a 96-well plate and then incubated for indicated time points at 37°C. An MTT solution was added to each well and incubated for 2 hours at 37°C. A lysis buffer (20% SDS and 50% dimethylformamide) was added, and the cells were incubated overnight at 37°C. The absorbance of the cell suspension was measured at 570 nm by a Tecan plate reader.

LIVE/DEAD assay
To investigate whether γ-tocotrienol could potentiate the apoptotic effects of capecitabine in gastric cancer cells, we used a LIVE/DEAD Cell Viability Assay Kit (Invitrogen), which is used to determine intracellular esterase activity and plasma membrane integrity. This assay uses calcine, a poly-anionic, green fluorescent dye that is retained within live cells, and a red fluorescent ethidium homodimer dye that can enter cells through damaged membranes and bind to nucleic acids but is excluded by the intact plasma membranes of live cells (20). Briefly, gastric cancer cells (5,000 per well) were incubated in chamber slides, pretreated with γ-tocotrienol for 4 hours, and treated with capecitabine for 24 hours. Cells were then stained with the assay reagents for 30 minutes at room temperature. Cell viability was determined under a fluorescence microscope by counting live (green) and dead (red) cells.

Flow cytometric analysis
To determine the effect on the cell cycle, cells were exposed to combination of γ-tocotrienol for 4 hours and treated with capecitabine for 24 hours. Thereafter cells were washed, fixed with 70% ethanol, and incubated for 30 minutes at 37°C with 0.1% RNase A in PBS. Cells were then washed again, resuspended, and stained in PBS containing 25 μg/ml propidium iodide (PI) for 30 minutes at room temperature. Cell distribution across the cell cycle was analyzed with a CyAn ADP flow cytometer (Dako Cytomation).

Gastric tumor model
All procedures involving animals were reviewed and approved by NUS Institutional Animal Care and Use Committee. Six-week-old athymic nu/nu female mice (Animal Resource Centre, Western Australia) were implanted subcutaneously in the right flank with SNU-5 cells (3 × 10^6 cells/100 μl saline). When tumors have reached 0.25 cm in diameter, the mice were randomized into the following treatment groups (n = 5 per group): (i) untreated control (corn oil, 100 μl daily); (ii) tocotrienol [1 mg/kg body weight, suspended in corn oil, intraperitoneal (i.p.) injection] 3 times/wk; (iii) capecitabine alone (60 mg/kg bodyweight, suspended in corn oil, twice weekly by gavage); and (iv) combination (tocotrienol, 1 mg/kg body weight, suspended in corn oil, i.p. injection) 3 times/wk and capecitabine (60 mg/kg body weight, suspended in corn oil, twice weekly by gavage). Therapy was continued for 4 weeks, and the animals were euthanized 1-week after. Primary tumors were excised and the final tumor volume was measured as V = 4/3πr^3, where r is the mean radius of the 3 dimensions (length, width, and depth). Half of the tumor tissue was fixed in formalin and embedded in paraffin for immunohistochemistry and routine hematoxylin and eosin (H&E) staining. The other half was snap frozen in liquid nitrogen and stored at −80°C.

Immunohistochemical analysis of gastric tumor samples
Solid tumors from control and various treatment groups were fixed with 10% phosphate-buffered formalin, processed, and embedded in paraffin. Sections were cut and deparaffinized in xylene and dehydrated in graded alcohol and finally hydrated in water. Antigen retrieval was carried out by boiling the slide in 10 mmol/L sodium citrate (pH 6.0) for 30 minutes. Immunohistochemistry was carried out following manufacturer instructions (DAKO LSAB kit). Briefly, endogenous peroxidases were quenched with 3% hydrogen peroxide. Nonspecific binding was blocked by incubation in the blocking reagent in the LSAB kit (Dako) according to the manufacturer’s instructions. Sections were incubated overnight with primary antibodies as follows: anti-p65, anti-COX-2, anti-VEGF, anti-MMP-9, anti-Ki-67, and anti-CD31 (each at 1:100 dilutions). Slides were subsequently washed several times in TBS with 0.1% Tween 20 and were incubated with biotinylated linker for 30 minutes, followed by incubation with streptavidin conjugate provided in LSAB kit according to the manufacturer’s instructions. Immunoreactive species were detected with 3,3′-diaminobenzidine (DAB) as a substrate. Sections were counterstained with the Gill hematoxylin and mounted under glass cover slips. Images were taken with an Olympus BX51 microscope (magnification, ×20). Positive cells (brown) were quantitated with the ImagePro plus 6.0 software package (Media Cybernetics, Inc.).

Preparation of nuclear extract from gastric tumor samples
Gastric tumor tissues (100 mg/mouse) from mice in the control and treatment groups were minced and incubated on ice for 30 minutes in 0.5 mL of ice-cold buffer A [10 mmol/L HEPES (pH 7.9), 1.5 mmol/L KCl, 10 mmol/L MgCl_2, 0.5 mmol/L dithiothreitol (DTT), and 0.5 mmol/L PMSF]. The minced tissue was homogenized with a Dounce
homogenizer and centrifuged at 16,000 × g at 4°C for 10 minutes. The resulting nuclear pellet was suspended in 0.2 mL of buffer B [20 mmol/L HEPES (pH 7.9), 25% glycerol, 1.5 mmol/L MgCl2, 420 mmol/L NaCl, 0.5 mmol/L DTT, 0.2 mmol/L EDTA, 0.5 mmol/L PMSF, and 2 μg/mL leupeptin] and incubated on ice for 2 hours with intermittent mixing. The suspension was then centrifuged at 16,000 × g at 4°C for 30 minutes. The supernatant (nuclear extract) was collected and stored at −80°C until used as previously described (35). Protein concentration was measured by the Bradford assay with BSA as the standard.

Measurement of NF-κB activation in gastric cancer cells and tumor samples
To determine NF-κB activation, we conducted DNA-binding assay by TransAM NF-κB p65 transcription factor assay kit (Active Motif) according to the manufacturer’s instructions and as described previously (20). Briefly, nuclear extracts from γ-tocotrienol–treated gastric cancer cell lines and tumor tissues were incubated in a 96-well plate coated with oligonucleotide containing the NF-κB consensus–binding sequence 5’-GGGACCTTCC-3’. Bound NF-κB was then detected by a specific primary antibody. An HRP-conjugated secondary antibody was then applied to detect the bound primary antibody and provided the basis for colorimetric quantification. The enzymatic product was measured at 450 nm with a microplate reader (Tecan Systems). Specificity of this assay was tested by the addition of wild-type or mutated NF-κB consensus oligonucleotide in the competitive or mutated competitive control wells before the addition of the nuclear extracts.

Statistical analysis
Statistical analysis was conducted by the Student t test and one way ANOVA. A P value of less than 0.05 was considered statistically significant.

Results
The purpose of this study was to determine whether γ-tocotrienol, a component of vitamin E (with chemical structure shown in Fig. 1A) might have a role in the treatment of gastric cancer either alone or in combination with capecitabine and if so, through what mechanism(s). For this, we used 4 different well-characterized human gastric cancer cell lines. To facilitate the monitoring of tumor growth in mice, one of these cell lines, SNU-5 was subcutaneously injected and used in the xenograft transplant model in mice.

γ-Tocotrienol inhibits the proliferation and potentiates the effect of capecitabine in gastric cancer cells in vitro
We first investigated the effect of γ-tocotrienol on the proliferation of 3 different gastric cancer cell lines. γ-Tocotrienol inhibited the growth of all 3 human gastric cancer cells (SNU-5, MKN45, and SNU-16) in a dose- and time-dependent manner (Fig. 1B). Whether γ-tocotrienol can potentiate the effect of capecitabine against these 3 cell lines was also examined. We used flow cytometric analysis and an esterase staining assay (LIVE/DEAD assay) to establish whether γ-tocotrienol can potentiate the apoptosis induced by capecitabine. As shown in Fig. 1C and D, the dose of γ-tocotrienol (10 μmol/L) or capecitabine (10 μmol/L) that had minimum effect on apoptosis alone produced enhancement of apoptosis when used in combination.

γ-Tocotrienol inhibits constitutive and capecitabine-induced NF-κB activation in gastric cancer cells
We next examined that how γ-tocotrienol potentiates the effects of capecitabine in gastric cancer cells. NF-κB has been shown to be constitutively expressed in gastric cancer and mediates resistance to apoptosis (5, 36, 37). Whether γ-tocotrienol induces downregulation of constitutive NF-κB activation in SNU-16 and SNU-5 cells was examined by using an ELISA-based DNA-binding assay. Results show that the treatment with γ-tocotrienol inhibited NF-κB expression in a dose-dependent manner (Fig. 2A and B). We further observed that chemotherapeutic drug capecitabine was also able to further induce NF-κB activation in a dose-dependent manner in MKN45 cells, with maximum activation observed at 25 μmol/L (Fig. 2C). Interestingly, γ-tocotrienol was also found to suppress capecitabine-induced NF-κB activation in a dose-dependent manner in MKN45 cells (Fig. 2D), thereby indicating that it is a potent modulator of both constitutive and inducible NF-κB activation in gastric cancer cells. Whether γ-tocotrienol can also modulate the expression of various NF-κB–regulated gene products was also examined. We found that the γ-tocotrienol suppressed the constitutive expression of antiproliferative (cyclin D1), antiapoptotic (Bcl-2), invasive/metastatic (ICAM-1, MMP-9, CXCR4), and angiogenic (VEGF) protein expression in a time-dependent manner in SNU116 cells (Fig. 2E). γ-Tocotrienol also induced the cleavage of PARP in SNU-16 cells (Fig. 2E). On the basis of these observations in vitro, we decided to study the effect of γ-tocotrienol and capecitabine either alone or in combination in an in vivo gastric cancer xenograft model.

γ-Tocotrienol potentiates the antitumor effects of capecitabine in a xenograft gastric cancer model in nude mice
We examined the therapeutic potential of γ-tocotrienol and capecitabine either alone or in combination on the growth of subcutaneously implanted human gastric cancer cells in nude mice. The experimental protocol is depicted in Fig. 3A. SNU-5 cells were implanted subcutaneously in the right flank of nude mice. When tumors have reached 0.25 cm in diameter after a week, the mice were randomized into 4 groups and started the treatment as per the experimental protocol. The treatment was continued for 4 weeks and animals were sacrificed after 5 weeks. We found that γ-tocotrienol alone when given at 1 mg/kg body weight significantly inhibited the growth of the tumor (P < 0.001 when compared with control; Fig. 3B and C). Capecitabine alone was also very effective (P < 0.001 when compared with control; P > 0.05 when compared with γ-tocotrienol...
alone group); and the combination of the 2 agents were more effective in reducing the tumor burden. The tumor volume in the combination of γ-tocotrienol and capecitabine group was significantly lower than γ-tocotrienol alone group (P < 0.001) or capecitabine alone group (P < 0.001) on day 35 (Fig. 3C and D).

γ-Tocotrienol inhibits CD31 and Ki-67 expression in gastric tumor tissues

While Ki-67–positive index is used as a marker for cell proliferation, the CD31 index is a marker for microvessel density. Whether γ-tocotrienol and capecitabine modulate these markers, was examined. Figure 4A shows that both γ-tocotrienol (P < 0.05) and capecitabine (P < 0.05) alone significantly downregulated the expression of Ki-67 in gastric cancer SNU-5 tissue and the combination of the 2 was most effective (P < 0.001). Similarly when examined for CD31, we found that both agents significantly reduced the CD31 expression as compared with control group and 2 together were most effective (P < 0.001 when compared with capecitabine alone; Fig. 4B).

Figure 1. γ-Tocotrienol inhibits the growth and proliferation, potentiates the apoptotic effects of capecitabine in gastric cancer cells in vitro. A, structure of γ-tocotrienol. B, MTT assay results showed dose-dependent suppression of cell proliferation in all 3 gastric cancer cell lines treated with γ-tocotrienol. Points, mean of triplicate; bars, SE. Data are a representative of 2 independent experiments. C and D, flow cytometric analysis and LIVE/DEAD assay results indicate that γ-tocotrienol (γ-Toco, 10 μmol/L) potentiates capecitabine (Cape, 10 μmol/L)-induced apoptosis in gastric cancer cells. Data indicated as percentage proportions of apoptotic gastric cancer cells for LIVE/DEAD assay. Values are mean ± SE of triplicate. Data are a representative of 2 independent experiments.
Figure 2. A and B, DNA-binding assay results showing that γ-tocotrienol suppresses the constitutive activation of NF-κB in SNU-16 and SNU-5 cells in a dose-dependent manner. SNU-16 and SNU-5 (1 × 10⁶) cells were treated with γ-tocotrienol (10, 25, and 50 μmol/L) for 4 hours, and nuclear extracts were prepared and assayed for NF-κB activation by ELISA-linked DNA-binding assay. C, MKN45 (1 × 10⁶) cells were treated with capecitabine (10, 20, and 25 μmol/L) for 4 hours, and nuclear extracts were prepared and assayed for NF-κB activation by ELISA-linked DNA-binding assay. D, MKN45 (1 × 10⁶) cells were pretreated with γ-tocotrienol (10, 25, and 50 μmol/L) for 4 hours, stimulated with capecitabine 25 μmol/L for 4 hours, and then the nuclear extracts were prepared and assayed for NF-κB activation by ELISA-linked DNA-binding assay. E, γ-tocotrienol suppressed the constitutive expression of gene products involved in proliferation, metastasis, and antipapoptosis in gastric cancer cells. SNU-16 (1 × 10⁶) cells were treated with 50 μmol/L γ-tocotrienol for the indicated time points, and Western blot analysis was conducted as described in Materials and Methods. *, P < 0.05.

γ-Tocotrienol enhances the effect of capecitabine in gastric cancer

We also evaluated the effect of γ-tocotrienol and capecitabine on NF-κB levels in gastric tumor tissue. Figure 5A, left shows that γ-tocotrienol either alone or in combination with capecitabine was quite effective in suppressing the constitutive expression of NF-κB in gastric cancer tissue. Capecitabine alone had no significant effect on constitutive NF-κB activation in gastric tissue. A Western blot analysis for p65 in extracts from tumor samples showed that γ-tocotrienol alone inhibited NF-κB (p65) activation (Fig. 5A, right).

NF-κB is known to regulate the expression of a number of proteins, including those involved in proliferation (cyclin D1, COX-2), invasion/metastasis (ICAM-1, MMP-9), and survival (Bcl-xL, survivin, and XIAP; ref. 38). Whether γ-tocotrienol and capecitabine can modulate the expression of these NF-κB-regulated gene products in tumor tissues was examined by Western blot analysis. We found that treatment with combination of γ-tocotrienol and capecitabine was effective in downregulating the overexpression of various gene products regulated by NF-κB (Fig. 5B) and also involved in various aspects of gastric cancer growth, survival, invasion, and metastasis.

Whether modulation of nuclear NF-κB, COX-2, VEGF, and MMP-9 can also be detected by immunohistochemical methods was also examined. As shown in Figure 6, these gene products were significantly downregulated in gastric tumor samples treated with γ-tocotrienol in combination with capecitabine. The downregulation was more impressive with either γ-tocotrienol or capecitabine alone. The immunohistochemical analysis results further supports the data obtained from Western blotting. These results collectively indicate that γ-tocotrienol suppresses the activation of NF-κB thereby inhibiting the expression of genes involved in proliferation, survival, invasion, and angiogenesis.
Clinical Cancer Research

Manu et al.

Discussion

Despite the major improvements in diagnosis and treatment regimens, gastric cancer remains one of the most lethal cancers, with less than 20% of patients surviving up to 5 years. Thus, novel agents that are nontoxic, efficacious, and can significantly enhance the effects of existing chemotherapeutic drugs are urgently needed. The aim of the present study was to investigate whether γ-tocotrienol, a component of vitamin E, could enhance the antitumor activity of capecitabine against human gastric cancer. We found that γ-tocotrienol suppressed the proliferation of various gastric cancer cell lines, potentiated capecitabine-induced apoptosis, and inhibited constitutively active and inducible NF-κB activation as well as NF-κB–regulated gene products. We also found that in a xenograft mouse model γ-tocotrienol effectively suppressed the growth of gastric cancer alone and also when used in combination with capecitabine.

We first observed that γ-tocotrienol can suppress the proliferation of various gastric cancer cell lines in a dose- and time-dependent manner. Our results are in part agreement with those of Sun and colleagues (17) who reported that γ-tocotrienol could suppress the proliferation of gastric adenocarcinoma SGC-7901 cells. The inhibitory effects of γ-tocotrienol were correlated with the DNA damage and activation as well as NF-κB–regulated gene products. We also found that in a xenograft mouse model γ-tocotrienol effectively suppressed the growth of gastric cancer alone and also when used in combination with capecitabine.

We first observed that γ-tocotrienol can suppress the proliferation of various gastric cancer cell lines in a dose- and time-dependent manner. Our results are in part agreement with those of Sun and colleagues (17) who reported that γ-tocotrienol could suppress the proliferation of gastric adenocarcinoma SGC-7901 cells. The inhibitory effects of γ-tocotrienol were correlated with the DNA damage and activation as well as NF-κB–regulated gene products. We also found that γ-tocotrienol effectively suppressed the growth of gastric cancer alone and when used in combination with capecitabine.

Specifically, we also found for the first time that γ-tocotrienol when used in combination with capecitabine, is...
highly effective in inducing apoptosis in gastric cancer cell lines. This is very intriguing because although γ-tocotrienol has been previously reported to induce apoptosis in SGC-7901 cells (16, 17), its effect in combination with chemotherapeutic agents like capcitabine has never been investigated before in gastric cancer. We observed that this effect may be mediated because of the downregulation of cell survival proteins such as Bcl-2 in gastric cancer. Interestingly, we also observed for the first time that both constitutive and capcitabine-induced NF-κB activation was suppressed by γ-tocotrienol in gastric cancer cells. These results are consistent with those previously reported with other dietary agents like curcumin (39) and phenethyl isothiocyanate (40). Also, γ-tocotrienol was found to downregulate the expression of various invasive, metastatic, and angiogenic gene products (ICAM-1, MMP-9, CXCR4, and VEGF) which may account for its recently reported inhibitory effects on invasion, metastasis, and angiogenesis (18, 41).

We found for the first time that the intraperitoneal administration of γ-tocotrienol alone inhibited the growth of human gastric tumors when examined in vivo in a xenograft nude mice model. Tumor growth was inhibited by more than 50% on treatment with γ-tocotrienol and capcitabine alone, respectively. Also, when the 2 agents were used in combination, they were found to be much more effective and potent. When examined for the mechanism by which γ-tocotrienol manifests its effects in the mice against gastric cancer, we found that the proliferation marker Ki-67 as well as microvessel density indicator CD31 was downregulated by γ-tocotrienol. Further investigation also revealed the downregulation of NF-κB and NF-κB–regulated cyclin D1, COX-2, survivin, Bcl-xL, XIAP, ICAM-1, and VEGF. All of these effects were further enhanced by capcitabine. γ-Tocotrienol has been used in combination therapy with several chemotherapeutic agents/targeted therapies such as statins in breast and colorectal cancers (42, 43), celecoxib in breast cancer (44), gemcitabine in pancreatic cancer (29), EGFR inhibitors erlotinib and gefitinib in breast cancer (45, 46), but so far its effects on gastric cancer mice models either alone or in combination has never been investigated before. Our results overall suggest for the first time that γ-tocotrienol has significant potential for the treatment of gastric cancer and its effects can be further enhanced by capcitabine. A number of clinical trials with tocotrienols in patients with pancreatic, prostate, and breast cancer are already in progress, and based on our results, well-designed clinical studies are required for potential translation of our preclinical findings also in patients with gastric cancer.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.
Grant Support
This work was supported by grants from NUS Academic Research Fund (grant R-184-000-170-112) and National Medical Research Council of Singapore (grants R-184-000-211-213 and R-184-000-201-275) to G. Sethi. A.P. Kumar was supported by grants from the National Medical Research Council of Singapore (grant R-713-000-124-213) and Cancer Science Institute of Singapore, Experimental Therapeutics I Program (grant R-713-001-011-271).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received September 26, 2011; revised January 18, 2012; accepted February 8, 2012; published OnlineFirst February 20, 2012.

References
8. Schimanski CG, Galle PR, Moehler M. Chemokine receptor CXCR4-7.
31. Yap WN, Zaiden N, Tan YL, Ngoh CP, Zhang XV, Wong YC, et al. Id1, inhibitor of differentiation, is a key protein mediating anti-tumor...


44. Shrode AB, Sylvester PW. Synergistic anticancer effects of combined gamma-tocotrienol and celecoxib treatment are associated with suppression in Akt and NFkappaB signaling. Biomed Pharmacother 2010;64:327–32.


First Evidence That γ-Tocotrienol Inhibits the Growth of Human Gastric Cancer and Chemosensitizes It to Capecitabine in a Xenograft Mouse Model through the Modulation of NF-κB Pathway


Updated version
Access the most recent version of this article at:
doi:10.1158/1078-0432.CCR-11-2470

Cited articles
This article cites 45 articles, 9 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/18/8/2220.full#ref-list-1

Citing articles
This article has been cited by 3 HighWire-hosted articles. Access the articles at:
http://clincancerres.aacrjournals.org/content/18/8/2220.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, use this link
http://clincancerres.aacrjournals.org/content/18/8/2220.
Click on "Request Permissions" which will take you to the Copyright Clearance Center’s (CCC) Rightslink site.