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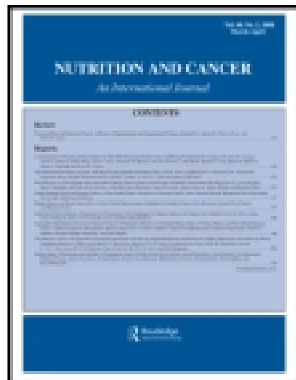

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Selective Growth Inhibition of Human Breast Cancer Cells by Graviola Fruit Extract In Vitro and In Vivo Involving Downregulation of EGFR Expression

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The epidermal growth factor receptor (EGFR) is an oncogene frequently overexpressed in breast cancer (BC), and its overexpression has been associated with poor prognosis and drug resistance. EGFR is therefore a rational target for BC therapy development. This study demonstrated that a graviola fruit extract (GFE) significantly downregulated EGFR gene expression and inhibited the growth of BC cells and xenografts. GFE selectively inhibited the growth of EGFR-overexpressing human BC (MDA-MB-468) cells (IC_{50} = 4.8 μg/ml) but had no effect on nontumorigenic human breast epithelial cells (MCF-10A). GFE significantly downregulated EGFR mRNA expression, arrested cell cycle in the G0/G1 phase, and induced apoptosis in MDA-MB-468 cells. In the mouse xenograft model, a 5-wk dietary treatment of GFE (200 mg/kg diet) significantly reduced the protein expression of EGFR, p-EGFR, and p-ERK in MDA-MB-468 tumors by 56%, 54%, and 32.5%, respectively. Overall, dietary GFE inhibited tumor growth, as measured by wet weight, by 32% ($P < 0.01$). These data showed that dietary GFE induced significant growth inhibition of MDA-MB-468 cells in vitro and in vivo through a mechanism involving the EGFR/ERK signaling pathway, suggesting that GFE may have a protective effect for women against EGFR-overexpressing BC.

INTRODUCTION

Breast cancer (BC) remains one of the most common female cancers (1). In 2010, there were an estimated 207,090 new cases and 39,840 deaths from BC in the United States (2). Considering the heterogeneity of BC and the limitations of current therapies due to severe side effects and drug resistance, there is an urgent need to explore alternative strategies to prevent and treat BC. Development of novel mechanism-based nutritional agents that could selectively target BC may offer an intriguing strategy for controlling the disease. Accumulating evidence suggests a strong effect of the diet or its components on BC development and progression, either through effects on hormonal status or via direct anti-tumor-promoting or anticarcinogenic effects (3). Epidemiologic studies have linked greater fruit and vegetable intake with lower risk of cancer (4). This beneficial effect is due in part to the fact that fruits and vegetables contain fiber, antioxidants, and other potentially antineoplastic compounds. Specific food bioactive components, notably sulfur-containing glucosinolates and green tea polyphenols, are associated with reduced risk of BC (5,6).

Graviola (Annonaceous muricata L.) is an Amazon fruit tree that grows in the tropics of North and South America and is also known as soursop and guanabana. Leaves and stems of graviola have been traditionally used as an herbal preparation for a variety of purported health-promoting effects, including supporting healthy cell growth and immune function (7). Graviola fruits have been widely consumed by indigenous people in fresh or processed forms for centuries. However, research on this fruit is extremely limited despite its regular consumption, and to date, there is no published study investigating the effect of graviola fruit on cancers.

The EGFR has been identified as a promising target for BC therapies. Binding of a ligand such as EGF or transforming

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growth factor α (TGFα) results in a signaling cascade that produces diverse effects, including cell migration, maturation, differentiation, metastasis, angiogenesis, and inhibition of apoptosis (9). High expression of EGFR is commonly considered the main mechanism by which EGFR signaling is increased in cancer cells (10). EGFR or its family members are highly expressed in a variety of human tumors, including BC (11). Its overexpression correlates inversely with estrogen receptor (ER) status in patients with BC (12) and is associated with poor prognosis and resistance to chemotherapy, hormone therapy, and radiation (13). Molecular inhibition of EGFR signaling by anti-EGFR monoclonal antibody or specific small molecule inhibitors has shown antitumor effects in clinical trials (14). This study determined the in vitro and in vivo effect of GFE treatment on the growth of MDA-MB-468 cells through a mechanism involving downregulation of EFGR expression and downstream effectors of the EGFR signaling pathway. MDA-MB-468 cells contain an amplified EGFR gene (8) and therefore serve as an excellent model in which to study anti-EGFR treatment.

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MATERIALS AND METHODS

Materials

Methyl thiazolyl tetrazolium bromide (MTT), propidium iodide (PI), and Triton X-100, 4′,6′-diamidino-2-phenylidple (DAPI) were purchased from Sigma Chemical Company (St. Louis, MO). An annexin V-FITC cell apoptosis kit was obtained from Zymed Laboratories, Inc. (San Francisco, CA). Reagents for RNA extraction and purification were purchased from Qiagen (Valencia, CA). Reagents for cDNA synthesis and quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) were purchased from Bio-Rad (Hercules, CA). Primers were purchased from Integrated DNA Technologies, Inc. (Coralville, IA). Animal diet was purchased from Dyets (Bethlehem, PA).

Graviola Fruit Extraction and Purification

The dried graviola fruit powder was obtained from Earthfruits (South Jordan, UT). GFE was prepared with 50% aqueous acetone extraction, which was subsequently filtered and lyophilized. Approximately 100–120 mg GFE was obtained from 10 g dried graviola fruit powder.

Cell Lines and Cell Culture

Human BC MDA-MB-468, MDA-MB-231, and MCF-7, and nontumorigenic breast epithelial MCF-10A cells were obtained from American Type Culture Collection (Manassas, VA). BC cells were maintained in DMEM/F12 supplemented with 10% heat-inactivated fetal bovine serum (FBS). MCF-10A cells were supplemented with 100 ng/ml cholera enterotoxin, 10 μg/ml insulin, 0.5 μg/ml hydrocortisol, 20 ng/ml epidermal growth factor, 5% horse serum, 10 mM HEPES (RPMI), and 2.2 g/L sodium bicarbonate, while MCF-7 cells were supplemented with 0.5 nmol/L estradiol and 5 μmol/L insulin. All cultures were maintained in growth medium in the presence of 100 units/ml penicillin and 100 μg/ml streptomycin at 37°C in a humidified atmosphere of 5% CO_2 in air as a monolayer culture in plastic culture plates. Growth medium was changed every 48 h, and cells were passed when they reached 85–95% confluence, as observed by light microscopy.

Cell Viability Assay (MTT Assay)

Cells were seeded in 96-well tissue culture plates (8.0×10^3 cells/well) and incubated with GFE for 24–96 h. After incubation, the growth medium was removed and cells were washed with HBSS and incubated for 4 h with MTT reagent solution. The color intensity of the formazan solution, which reflects the number of cells under the specific growth conditions, was measured at 570 nm using a Victor^3 multilabel plate reader (Perkin Elmer, Waltham, MA). The cell density of treatment groups was expressed as percentage of the control.

Caspase-3 Activity

Following GFE treatments, MDA-MB-468 cells were harvested in cell lysis buffer and incubated on ice for 1 h. After centrifugation at $11,000 \times g$ for 30 min, the supernatants were collected and protein concentration and caspase-3 activity immediately measured using a detection kit following the manufacturer's protocol (TruPoint Caspase-3 Assay Kit, Perkin Elmer Life and Analytical Sciences, Norton, OH).

Cell Cycle Analysis

MDA-MB-468 cells were treated by 0, 5, 25, 50, or 100 μg/ml GFE for 48 h. After trypsinization, cells were collected, washed, and suspended in 0.5 ml PBS (1×10^6 cells/ml). The cell suspension was added to 4.5 ml of 70% ethanol and stored at 4°C for 2 h. After centrifugation, cells were washed with PBS and resuspended in 1 ml PI/ Triton X-100 staining solution with DNase-free RNase A at 37°C for 30 min before analysis by the FACS Aria flow cytometer (BD Bioscience, San Jose, CA). The experiments were conducted in triplicate.

Analysis of EGFR mRNA Expression Using qRT-PCR

After collecting 1.5×10^6 of MDA-MB-468 cells which were treated by 5 μg/ml (low) and 100 μg/ml (high) GFE for 48 h, total RNA was isolated using the RNeasy Mini Kit (Qiagen Company, Valencia, CA) and quantified by UV absorbance. cDNA was generated using 10 ng of RNA and iScript Reverse Transcription Reagents as described in the manufacturer's protocol (Bio-Rad). Primers were designed using Beacon Designer 5 (Premier Biosoft International, Palo Alto, CA). The oligonucleotide primers specific for EGFR and GAPDH (EGFR: forward primer, 5′-CCGTCGCTATCAAGG AATTAAG-3′; reverse primer, 5′-GTGGAGGTGAGGC AGATGG-3′; GAPDH: forward primer, 5′-TTGGTATCGTGGAAGGACTC-3′; reverse primer, 5′-TAGAGGCAGGGATGATGTTC-3′) were used. PCR and analysis of PCR products were performed using the iCycler (Bio-Rad) and the SYBR-green detection system. Data were analyzed using a comparative threshold cycle (Ct) method.

Each sample was run in triplicate in separate tubes to permit quantification of target genes normalized to controls, GAPDH.

Athymic Nude Mice

Five-wk-old female athymic BALB/c (nude) mice were purchased from Charles River Laboratories (Wilmington, MA). During the study, the mice were maintained under the standard light/dark cycle (12-h light, 12-h dark).

Animal Treatment and Analysis of Tumor Growth

After 1 wk of acclimatization, MDA-MB-468 cells [1×10^5 cells/40 μl of Matrigel (Collaborative Biomedical Products, Bedford, MA), 4 spots/mouse] were injected into the back of the athymic mice. Mice were randomly divided into 2 groups, MDA-MB-468 control and 200 mg GFE/kg diet ($n = 6$), and dietary treatment began. The selected daily GFE dose was equivalent to 500–700 g of fresh fruit (2–3 fruits), reflecting rational daily human consumption levels. American Institute of Nutrition 93 growth diet (AIN93G) semipurified diet (Dyets, Bethlehem, PA) was selected as a base diet for control mice as it has been established that it meets all the nutritional requirements of mice (15). Treatment animals were fed AIN93G diet plus GFE (200 mg/kg diet) for 5 wk until the average tumor surface area of the control group reached 90.8 mm^2. Tumor surface area and body weight were measured weekly, and surface area was determined using the formula [length/2 $\times$ width/2 $\times$ π] (16). Food intake was measured throughout the study. At termination, tumor wet weight was measured. Tumors and blood samples were collected for further analysis. Animal husbandry, care, and experimental procedures were conducted in compliance with the Principles of Laboratory Animal Care NIH guidelines, as approved by the Institutional Animal Care and Use Committee (IACUC) at Virginia Tech (06–010-HNFE).

Western Blotting Analysis

The dietary GFE-induced changes in protein expression of EGFR, phosphorylated-ERK (p-ERK), and p-Akt were examined. Blots were stripped and reprobed with antibody that recognizes total ERK or Akt. Frozen tumors (4 tumors/control and 6 tumors/GFE) were pulverized with mortar and pestle in liquid N_2 and lysed and homogenized in radioimmunoprecipitation assay (RIPA) buffer. Homogenates were centrifuged at $10{,}000 \times g$ for 10 min at 4°C and supernatant was collected for analysis. Protein from tumors (10–15 μg) was loaded into a 7.5% gradient gel, electrophoresed at 100 V for 1.5 h, and transferred to nitrocellulose membrane at 100 V for 1 h. The membrane was blocked for 1 h in 5% milk-TBST at room temperature, washed 3× with TBST, incubated overnight at 4°C in primary antibody (EGFR, p-ERK, and p-Akt, Santa Cruz; Santa Cruz, CA) washed 3× with TBST, incubated for 1 h at RT in secondary antibody (goat antirabbit-HRP, bovine antimouse-HRP, goat antirabbit-HRP, respectively) (Santa Cruz Biotechnology, Santa Cruz, CA), and activated with SuperSignal West Femto Maxiumum Sensitivity Substrate (Thermo Fisher Scientific, Inc., Rockford, IL). The membrane was then exposed to film and developed manually. Protein bands were analyzed using Image J (NIH, Bethesda, MD), and the bands from each film were normalized to β-actin (1°Ab goat, 2°Ab bovine antigoat-HRP).

Statistical Analyses

Values are expressed as the mean ± SEM. Data from in vitro experiments, tumor wet weight, Western blot analysis, body weight gain, and food intake were analyzed using 1-way or repeated-measures analysis of variance according to the characteristics of the data set using the SAS program (SAS, Cary, NC). If the overall treatment F-ratio was significant ($P < 0.05$), the differences between treatment means were tested with Tukey's multiple comparison test. Differences were considered to be significant if $P < 0.05$.

RESULTS

GFE Treatment Selectively Inhibited Growth of Human BC Cells

The inhibitory effect of GFE treatments (96 h) on the growth of BC cells and nontumorigenic breast cells is shown in Fig. 1. Among the BC cells, MDA-MB-468 cells were extremely sensitive to GFE treatment; the cell growth was inhibited by 29.5%, with the GFE concentration as low as 0.5 μg/ml, an effect that was highly dose-dependent. The IC_{50} of GFE for MDA-MB-468 cells was 4.8 μg/ml. In contrast, other BC cells, such as MDA-MB-231 and MCF-7 cells, were less sensitive to GFE treatments. GFE treatments did not affect the growth of nontumorigenic MCF-10A cells, even with a concentration as high as 200 μg/ml (Fig. 1A). These observations suggest that GFE-induced growth inhibition is not only cancer-specific but also highly selective against the different BC cells. Therefore, MDA-MB-468 cells were selected for further GFE investigation.

Time-Dependence of GFE Effect on the Growth of MDA-MB-468 Cells

As shown in Fig. 1B, the time-dependence of GFE effect on the growth of MDA-MB-468 cells was more significant when the cells were treated with the lower concentrations of GFE (1 and 2 μg/ml). When the BC cells were treated with the higher concentrations of GFE (5–25 μg/ml), the cell growth was predominately inhibited in the first 24-h incubation; the relative cell density remained stable when time was extended to 96 h. In Fig. 1C, the cell density of MDA-MB-468 was remarkably decreased, and numerous cells shrank after 48-h treatment with 25 μg/ml GFE.

GFE Treatment Induced Apoptosis in MDA-MB-468 BC Cells

The extent of apoptosis was determined by measuring the level of caspase-3 activation following GFE exposure (Fig. 2A). GFE treatment activated the caspase-3 activity in a dose-dependent manner. The enzyme activity was increased by 17.8%

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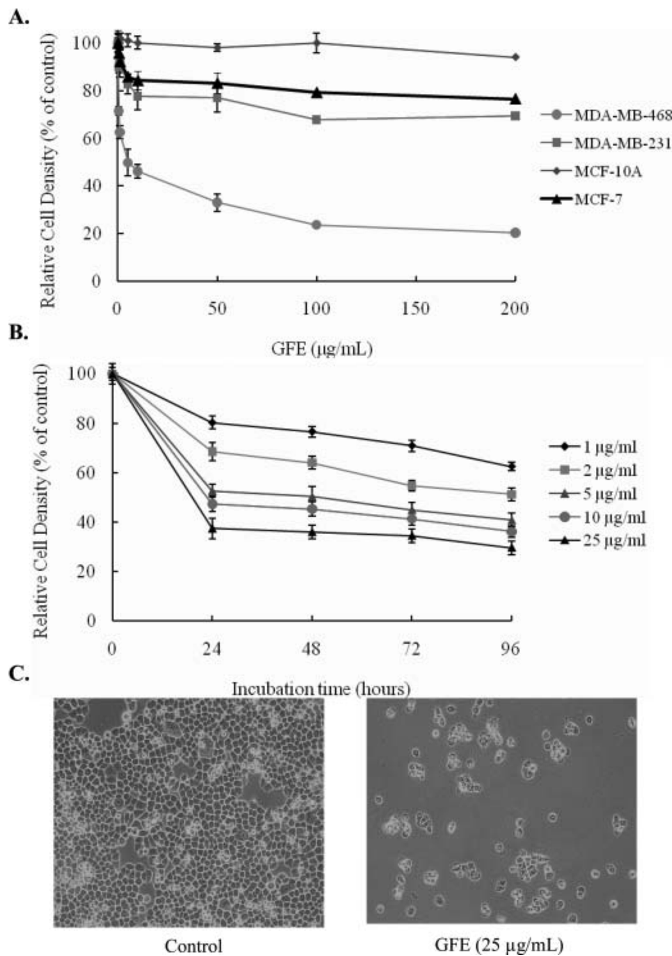

FIG. 1. Graviola fruit extract (GFE) treatment selectively inhibited the growth of human BC cells. A: Dose-response of GFE on human BC cells (MDA-MB-231, MDA-MB-468, and MCF-7) and nontumorigenic MCF-10A cells. Cells were grown in experimental growth medium of GFE for 96 h ($n = 3$). B: Time- and dose-effects of GFE on MDA-MB-468 cells. Cells were grown in growth medium containing GFE for durations indicated. Data are expressed as the percentage of control cells, mean ± SEM ($n = 3$). C: Representative image of MDA-MB-468 cells 48 h after treatment with GFE (25 μg/mL) or vehicle. Cells were seeded at 5×10^4 cells/mL on a 48-well plate for 48 h with the GFE treatment of 0 and 25 μg/mL ($n = 3$).

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and 91.9% after treatment with GFE at 12.5 and 200 μg/ml media, respectively ($P < 0.05$).

GFE Treatment Induced G1 Phase Arrest in MDA-MB-468 BC Cells

To further elucidate the mechanisms of GFE-induced growth inhibition that may accompany the apoptosis, we examined the effect of GFE treatment on cell cycle distribution. Concomitant with the growth inhibitory effect, GFE induced G0/G1-phase arrest in a dose-dependent manner (Fig. 2B and 2C). The G0/G1 population was increased by 3%, 5%, and 6% after 24-h GFE treatments at 25, 50, 100 μg/ml, respectively. This increase in the G0/G1 population paralleled a concomitant decrease in the S and G2/M populations. Concurrently, the apoptotic cell population of MDA-MB-468 cells was significantly increased after 24-h GFE treatment. The G1/S ratio has been used as an index of G1 arrest (17). The G1/S ratios of the GFE-treated cells were significantly higher than the control ($P < 0.05$) and a constant increasing pattern of the G1/S ratio was observed with increasing GFE concentrations.

GFE Treatment Downregulated mRNA Expression of EGFR in MDA-MB-468 Cells

MDA-MB-468 cells contain an amplified EGFR gene, and consequently these cells show very high expression levels of EGFR (1.5×10^6 receptor molecules/cell) (8). Previous experiments have shown that MDA-MB-468 cells were most sensitive to GFE treatment, followed by MDA-MB-231 and MCF-7 cells. Western blot was conducted to compare EGFR protein levels in these cell lines. Ten μg of total protein were separated by 7.5% SDS-PAGE and transferred to nitrocellulose membranes. The proteins were probed with a rabbit anti-EGFR polyclonal antibody. The ratio of EGFR/β-actin was 4.76 ± 0.62 for MDA-MB-468, 0.58 ± 0.09 for MDA-MB-231, and 0.02 ± 0.01 for MCF-7. The result indicated that the selective GFE-induced growth inhibition of MDA-MB-468 cells may be associated with the inhibition of EGFR signaling. Using qRT-PCR to measure EGFR mRNA level, it was observed that GFE treatment at 5 and 100 μg/ml significantly downregulated the EGFR gene expression by 30 ± 3.5% and 54 ± 8.4%, respectively ($P < 0.05$).

Dietary GFE Treatment Significantly Inhibited the Growth of MDA-MB-468 Tumors Implanted in Athymic Mice

The promising in vitro results prompted us to further examine, via a dietary intervention experiment, whether GFE exerts similar anticancer effects in vivo. After 5 wk of dietary GFE treatment, average tumor surface areas were 90.82 ± 9.15 mm^2 in the control group and 80.97 ± 5.62 mm^2 in the GFE (200 mg/kg diet) group, which is not a significant difference. However, we observed a significant reduction of tumor wet weight in the GFE group (reduced by 32%). Average tumor weight was 253.3 ± 54.7 mg in the control group, and 171.2 ± 23.2 mg in the GFE group ($P < 0.05$). There was no significant difference in food intake between the control and the GFE treatment group (data not shown). However, there was a significant difference in body weight gain between the 2 groups. Body weight gain was calculated using the following formula: body weight gain = (body weight at Week 5 – tumor weight) – (body weight at Week 1). Average body weight gain was 2.6 ± 0.4 g for the control group, and 0.5 ± 0.4 g for the GFE group, respectively ($P < 0.05$).

Dietary GFE Treatment Significantly Reduced the Protein Expression of EGFR, p-ERK, and p-EGFR in Tumors

As shown in Figs. 3 and 4, dietary GFE treatment inhibited the protein expression of EGFR, p-ERK, and p-EGFR in MDA-MB-468 tumors by 56%, 54%, and 33%, respectively

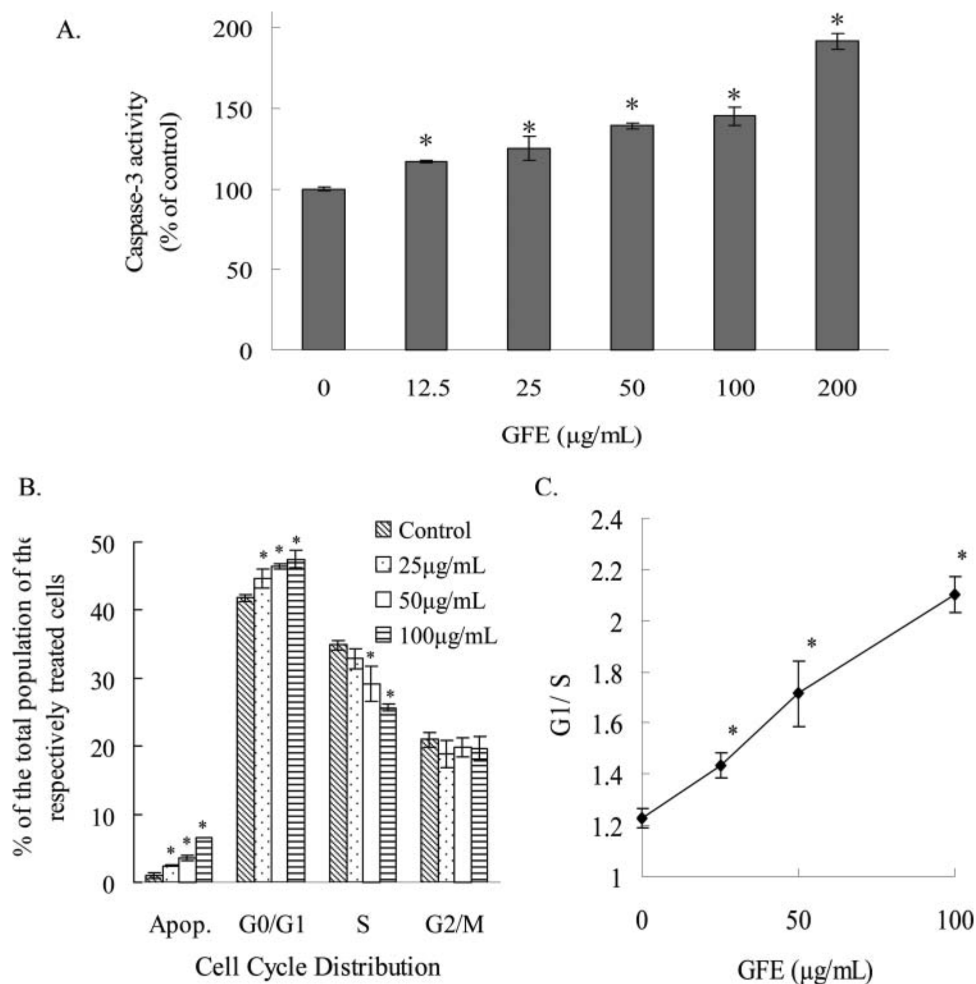

FIG. 2. Graviola fruit extract (GFE) treatment induced apoptosis in MDA-MB-468 BC cells. A: Caspase-3 activity as a function of different concentrations of GFE. Cells were treated with various concentrations of GFE for 48 h and extracted. Data are expressed as a percentage of control cells, mean ± SEM (n = 3), and asterisks indicate a statistically significant difference compared to control cells ($P < 0.05$). B and C: Cell cycle distribution after exposure to different concentrations of GFE. Cells were grown in experimental media containing 0, 25, 50, or 200 μg/ml of GFE for 24 h. Data are expressed as a percentage of the total population of cells receiving the respective treatments, mean ± SEM (n = 3). Asterisks indicated a statistically significant difference compared to control cells ($P < 0.05$).

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($P < 0.05$). However, no change was detected in the protein expression of p-Akt in tumors.

DISCUSSION

Identification of novel, targeted chemotherapeutics that can selectively inhibit tumors is of major importance in efforts to reduce the burden of BC. One such strategy to control BC growth and metastasis could be its prevention and treatment by phytochemicals, present in diets that specifically target BC cells. GFE might be such a novel dietary agent that was initially identified in our laboratory with anticancer potential through our preliminary screening of hundreds of food extracts and compounds on growth inhibition of BC cells. GFE showed unusual selective cytotoxicity to specific types of BC cells, suggesting that GFE may selectively target specific mechanisms in certain BC cells such as MDA-MB-468 cells.

To reveal the potential mechanisms governing the GFE-induced growth inhibition of MDA-MB-468 cells, we first examined whether GFE induced apoptosis and/or cell cycle arrest. Apoptosis regulates tissue homeostasis and is a critical mechanism for cancer chemoprevention and chemotherapy (18). In BC, cells become resistant to apoptosis partially due to disruptions of apoptotic signaling pathways and changes in the expression of enzymes associated with tumor resistance (19). In this study, we found that GFE treatment induced a dose-dependent increase in caspase-3 activity (Fig. 2A) and apoptotic cell death.

Cell cycle deregulation is closely associated with apoptosis, and disruption of the cell cycle may eventually lead to apoptotic death. Thus, inhibition of deregulated cell cycle progression in cancer cells is another effective strategy to control tumor

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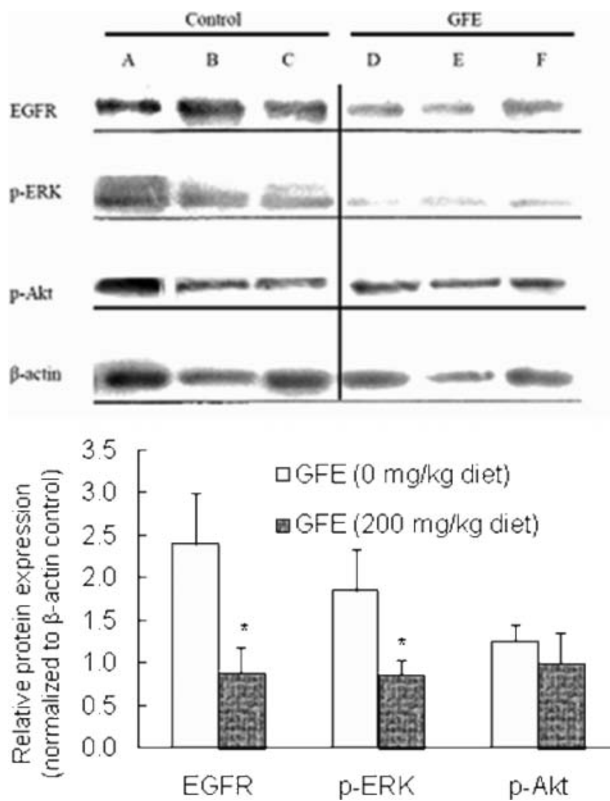

FIG. 3. Dietary graviola fruit extract (GFE) inhibited EGFR and p-ERK protein expression in tumors. Tumors in the control group (A, B, C) and GFE group (D, E, F) were analyzed using Western blot analysis (4 and 6 tumors from the control and GFE groups, respectively). Ratio of the target protein to the standard protein expression level (bars ± SEM) is displayed on the Y axis. β-actin was used as a standard for the quantity analysis. Asterisks indicated a statistically significant difference compared to control cells ($P < 0.05$).

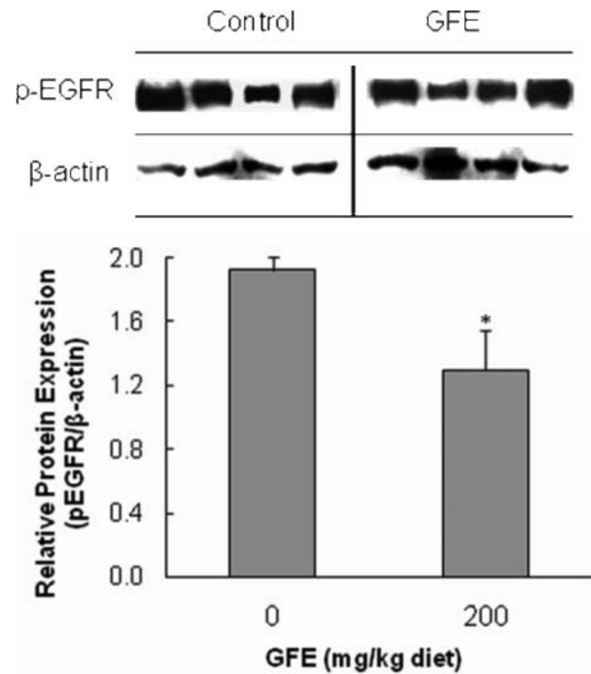

FIG. 4. Dietary graviola fruit extract (GFE) inhibited p-EGFR protein expression in tumors. Tumors were analyzed using Western blot analysis (4 and 6 tumors from the control and GFE groups, respectively). Numbers on the Y axis represent the ratio of the target protein to the standard protein expression level (bars ± SEM). β-actin was used as a standard for the quantity analysis. Asterisks indicated a statistically significant difference compared to control cells ($P < 0.05$).

growth (20). Chemotherapeutic agents often induce cell cycle arrest at the G0/G1 or G2/M phases (17,21). Our data showed that the increase of G0/G1 phase cells was accompanied by a significant decrease of S-phase cells and moderate decrease of G2/M phase cells, indicating that GFE treatment brought about a blockage effect at the G1/S transition and induced dose-dependent G0/G1 cell cycle arrest (Fig. 2B). These observations suggest that GFE-induced growth inhibition of MDA-MB-468 cells is partly attributable to its induction of apoptosis and G0/G1 cell cycle arrest. However, the specific mechanism of the GFE treatment on the cell cycle machinery and expression of several related proteins deserves further investigation.

MDA-MB-468 cells exhibit EGFR gene amplification (23). The extreme sensitivity of MDA-MB-468 cells to GFE treatment prompted us to examine whether GFE can target EGFR signaling. Indeed, we found that incubating MDA-MB-468 cells with GFE resulted in significant downregulation of EGFR mRNA expression in both lower (5 μg/ml) and higher concentrations (100 μg/ml) of GFE. Molecular inhibition of EGFR signaling has been shown to induce apoptosis and block cell cycle progression in cancer cells (9). GFE may also be acting in this manner against MDA-MB-468 cells. We also noted that there was no significant difference in tumor surface area between the control and GFE groups during the feeding study. It is possible that tumor volume and mass are not directly proportional because tumor growth is three-dimensional, and tumors consist of tumor cells, inflammatory cells, edema, fibrosis, or necrosis. Therefore, tumor wet weight at the termination would provide a more accurate endpoint compared to surface area.

Activation of EGFR occurs frequently in both benign and malignant hyperproliferative BC and triggers a cascade of downstream intracellular signaling pathways that contribute to tumorigenesis (9,24–26). This activation involves at least two major pathways: the highly conserved Ras/mitogen activated protein kinase (MAPK)-dependent pathway [Ras/Raf/extracellular signal-regulated kinase (MEK)/extracellular signal-regulated kinase (ERK)] and the phosphatidylinositol 3-kinase (PI3-K)-dependent pathway [PI3K/phosphatase and v-akt murine thymoma viral oncogene homolog (Akt)/mammalian target of rapamycin (mTOR)]. The activation of the Ras/Raf/MEK/ERK pathway results in cell survival, proliferation, migration, angiogenesis, and inhibition of apoptosis (24). We examined whether the GFE-induced tumor growth inhibition was attributable to modulation of EGFR and these 2 major signaling pathways, through measuring the protein expression of EGFR, p-EGFR, p-ERK, and p-Akt in tumors. Dietary GFE, at a rational human

exposure level (200 mg/kg diet), significantly inhibited protein expression of EGFR and p-EGFR and phosphorylation of ERK but not of Akt. These results suggested that dietary GFE treatment may downregulate EGFR expression and Ras/Raf/MEK/ERK pathways in vivo resulting in tumor inhibition.

In summary, this is, to the best of our knowledge, the first report demonstrating that a dietary agent at rational human exposure levels significantly downregulates EGFR expression and inhibits the growth of EGFR-overexpressing human BC cells both in vitro and in vivo. GFE selectively inhibited the growth of MDA-MB-468 cells without any effect on nontumorigenic cells, suggesting that GFE possesses selective antitumor properties. We further confirmed the anti-EGFR and antitumor activities of GFE in vivo. Dietary GFE significantly inhibited tumor growth in nude mice xenografts, suggesting that GFE might be developed as a novel mechanism-based dietary agent for the prevention and treatment of specific human BC. Further investigation of the above observed inclinations and active components in GFE is essential to establish solid grounds for the possible future utilization of this dietary extract as a chemopreventive agent.

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