Ginger Extract Inhibits Human Telomerase Reverse Transcriptase and c-Myc Expression in A549 Lung Cancer Cells

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ABSTRACT The rhizome of ginger (Zingiber officinale Roscoe) has been reputed to have many curative properties in traditional medicine, and recent publications have also shown that many agents in ginger possess antitumor properties. Here we show that the ethyl acetate fraction of ginger extract can inhibit the expression of the two prominent molecular targets of cancer, the human telomerase reverse transcriptase (hTERT) and c-Myc, in A549 lung cancer cells in a time- and concentration-dependent manner. The treated cells exhibited diminished telomerase activity because of reduced protein production rather than direct inhibition of telomerase. The reduction of hTERT expression coincided with the reduction of c-Myc expression, which is one of the hTERT transcription factors; thus, the reduction in hTERT expression might be due in part to the decrease of c-Myc. As both telomerase inhibition and Myc inhibition are cancer-specific targets for cancer therapy, ginger extract might prove to be beneficial as a complementary agent in cancer prevention and maintenance therapy.

KEY WORDS: • cancer therapy • c-Myc • ginger extract • human telomerase reverse transcriptase • Zingiber officinale

INTRODUCTION

The rhizome of ginger (Zingiber officinale Roscoe), one of the most widely used spices in food, has been reputed to have many curative properties in traditional medicine. Its medicinal uses range from the treatment of various gastrointestinal ailments, such as nausea, vomiting, abdominal discomforts, and diarrhea, to the treatment of arthritis, rheumatism, pain, and muscle discomfort and to the alleviation of various cardiovascular diseases and metabolic diseases.1-3 In addition to these well-documented properties of ginger, recent scientific studies have revealed that ginger also possesses anticancer properties in a wide range of experimental models.4,5 Telomerase is an attractive target for cancer therapy and cancer prevention because of its essential role in the immortal characteristics of most cancer cells.6 Without telomerase, telomeres of normal somatic cells are shortened every cell division, and once a telomere in a cell is shortened to a critical length, the cell is triggered to cellular senescence.7 The reactivation of telomerase, which is found in approximately 90% of all malignant tumors,8 maintains the telomere length and therefore prevents cancer cells from the replicative senescence. Telomerase is a reverse transcriptase that uses its internal RNA template to add telomeric repeats at the 3'-end of each telomere.9 Human telomerase is a multisubunit ribonucleoprotein complex in which the isolated catalytically active enzyme consists of three components: the human telomeric RNA, the human telomerase reverse transcriptase (hTERT), and dyskerin.10 Although human telomeric RNA and hTERT are both essential components for telomerase activity and telomere maintenance,11 transcriptional regulation of hTERT seems to be a predominant mechanism of controlling telomerase activity.12,13 The hTERT promoter is complex and contains many sites for a variety of transcription factors.11,14 One of these transcription factors, c-Myc, together with Max, binds to the E-box on the hTERT promoter, leading to the activation of hTERT expression.15 Myc proteins are well established as the master regulators of genes involved in protein synthesis, cellular proliferation, and carcinogenesis. Deregulation of Myc might initiate carcinogenesis by eliciting cooperative effects on cell growth, cell cycle progression, and genome instability.16 The Myc–Max–Mad network regulates gene transcription by distinct interactions among their members; while Myc–Max heterodimers activate specific gene transcription, Mad–Max heterodimers inactivate it by interacting with the same recognition element (E-box) at the gene promoter.17 Max is
stable and constitutively expressed, but Max-interacting proteins (e.g., Myc and Mad) are short-lived and highly regulated, suggesting that the gene regulation is largely dependent on those Max-interacting proteins. The increase in Myc level occurs through both transcriptional and posttranscriptional mechanisms in response to induction by a wide range of growth factors, cytokines, and mitogens. Therefore, Myc has been investigated as a feasible cancer therapeutic target.

In the present study, we report for the first time that the ethyl acetate fraction of ginger rhizome extract inhibits hTERT expression in A549 lung carcinoma cells, in parallel with the reduction of c-Myc expression.

**MATERIALS AND METHODS**

**Materials**

The plant material was collected in March 2008 from Lampang Province, Thailand, and was identified as Z. officinale Roscoe. A voucher specimen (BFK number 118527) is deposited in the Forest Herbarium, National Park, Wildlife and Plant Conservation Department, Ministry of Natural Resources and Environment, Bangkok, Thailand. The human lung carcinoma cell line A549 was obtained from the American Type Culture Collection (Rockville, MD, USA). The hTERT antibody (600-401-252) was purchased from Rockland Immunochemicals (Gilbertsville, PA, USA), and the e-Myc antibody (3C117) was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The SuperSignal® West Pico chemiluminescent substrate was obtained from Thermo Fisher Scientific (Rockford, IL, USA). Sulfur dockerine B was purchased from Sigma (St. Louis, MO, USA). The 6-gingerol was purchased from Sigma and China Aroma Chemical (Hangzhou, China). Taq DNA polymerase was purchased from Vivantis Technologies (Subang Jaya, Malaysia). All oligonucleotides and fluorescent-labeled oligonucleotides were obtained from Bio Basic Inc. (Markham, ON, Canada). All other common chemicals and culture media were of molecular biology grade.

**Preparation of Z. officinale extracts**

The air-dried and finely powdered rhizomes of Z. officinale (828.52 g) were extracted sequentially with hexane, ethyl acetate, acetone, and methanol (soaked in 7 L of solvent for 3 days, repeated five times for each solvent). Each fraction was collected, filtered, and evaporated to dryness under reduced pressure to afford 65.1, 48.4, 17.6, and 27.2 g of residues, respectively. These fractions were dissolved in dimethyl sulfoxide prior to use and stored at 4°C.

**Cell lines**

The A549 cells were grown in RPMI 1640 medium (Gibco, BRL, Grand Island, NY, USA) supplemented with 10% fetal bovine serum, penicillin (50 units/mL), and streptomycin (50 μg/mL) as a monolayer at 37°C in a humidified CO2 (5%) incubator.

**In vitro growth inhibition assays**

The growth inhibition of each fraction was determined using the sulfurondamine B assay according to a published protocol. The 50% growth inhibitory concentration (IC₅₀) was determined from the dose–response relationship between the ginger extract concentration and the percentage of growth inhibition. The results represent the means of three independent experiments.

**Semi-quantitative reverse transcription–polymerase chain reaction analysis**

A549 cells (3.0×10⁵ cells) were first allowed to grow on a six-well tissue culture plate for 24 hours before being treated with the indicated concentration of Z. officinale extract for another 24 hours at 37°C in a humidified CO₂ (5%) incubator. The total RNA purified from the treated cells was converted into cDNAs using oligo(dT)₁₂ primer and AMV reverse transcriptase (Fermentas, Hanover, MD, USA) according to the manufacturer’s instructions. The subsequent polymerase chain reaction (PCR) was carried out in a thermocycler. PCR products were electrophoretically separated on 1% agarose gel and visualized by ethidium bromide staining. The primer sequences of the genes of interest, the product sizes, and the number of cycles used in reverse transcription (RT)–PCR analysis are summarized in Table 1. The primer sequences were taken from a previously published article. The specific PCR cycle used for each gene was carefully chosen so that the intensity of the detected PCR product was proportional to the initial amount of cDNA in the reaction (Supplementary Fig. S1; Supplementary Data are available online at www.liebertonline.com/).

**Western blot analysis**

The cells were treated in the same manner as in the RT-PCR analysis. After treatment, the cells were lysed in ice-cold lysis buffer (10 mM Tris-HCl [pH 7.5], 1 mM MgCl₂, 1 mM EGTA, 0.5% CHAPS, 10% glycerol, and 5 mM β-mercaptoethanol). The protein (30 μg) from the cell lysates was separated by 8% sodium dodecyl sulfate–polyacrylamide gel electrophoresis and was transferred onto a nitrocellulose membrane by electroblotting. The membrane was probed with the indicated primary antibody. Following enzyme-linked secondary antibody incubation, the signal was detected by enhanced chemiluminescence (Thermo Fisher Scientific) and captured on Kodak (Rochester, NY, USA) X-ray film.

**Modified fluorescent telomerase repeat amplification protocol assay**

We investigated the effect of Z. officinale extract (ethyl acetate fraction) on telomerase activity by evaluating two phenomena: (a) the cellular effect on telomerase activity and (b) the direct effect on telomerase activity in a cell-free system.
TABLE 1. THE PRIMER SEQUENCE, PRODUCT SIZE, AND NUMBER OF POLYMERASE CHAIN REACTION CYCLES USED IN SEMIQUANTITATIVE REVERSE TRANSCRIPTION–POLYMERASE CHAIN REACTION ANALYSIS

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer sequence</th>
<th>Product size</th>
<th>Annealing temperature (°C)</th>
<th>Number of cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>hTERT</td>
<td>5'-GCGTTGACGCTGACCTTGCTA-3'</td>
<td>457, 275</td>
<td>62</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>5'-CCGAAACGCTGACCTTGCTGTC-3'</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>hTR</td>
<td>5'-GAGGGCCAGGAGGCACGGATGCTCTGTC-3'</td>
<td>111</td>
<td>60</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>5'-GTTGCTCTAGAAGCTGAGGGTGTA-3'</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>hTEP1</td>
<td>5'-TCAAGCCAAAGGCTGACCTTGAG-3'</td>
<td>264</td>
<td>60</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>5'-CCCCGACTGTAATCTTCTACGC-3'</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TRF1</td>
<td>5'-TGGGCGGTCGTAAGGATGAG-3'</td>
<td>421</td>
<td>62</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>5'-GCTGTCATTTCCAGGGGTA-3'</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TRF2</td>
<td>5'-AGTCAATCTGAGGCTGCTC-3'</td>
<td>636</td>
<td>60</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>5'-CGTGGTCTGTCGGCGTTAT-3'</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GAPDH</td>
<td>5'-CCACAGTCCATGCCACATC-3'</td>
<td>450</td>
<td>60</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>5'-CCACGACACCTGCTTGCTGTA-3'</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>c-Myc</td>
<td>5'-TAAATTCCAGGCGACGGAGCAG-3'</td>
<td>290</td>
<td>60</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>5'-GTCCCCAATAATGGGGCAATTA-3'</td>
<td></td>
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</tbody>
</table>

GAPDH, glyceraldehyde 3-phosphate dehydrogenase; hTEP1, human TEP1; hTERT, human telomerase reverse transcriptase; hTR, human telomeric RNA.

Cellular effect on telomerase activity. A549 cells were treated with various concentrations of Z. officinale extract for 24 hours. The cells were lysed with 200 μL of CHAPS lysis buffer (10 mM Tris-HCl [pH 7.5], 1 mM MgCl₂, 0.1 mM EGTA, 5 mM β-mercaptoethanol, 10% glycerol, protease inhibitor cocktail, and 200 units/mL RNase inhibitor), and the supernatant was collected and quantified for protein concentration by the Bradford assay (Bio-Rad, Hercules, CA, USA). The 200 ng of protein from this supernatant served as a source of telomerase. A modified fluorescent telomerase repeat amplification protocol (TRAP) assay was performed according to a published protocol; this method prevents the shortening and lengthening of the original telomerase products as normally seen from a standard TRAP assay. Our modification used a fluorescent primer rather than radioactive nucleotide incorporation. In brief, the 200 ng sample of crude telomerase from the cells treated with Z. officinale was incubated with 45 μL of telomerase reaction buffer (20 mM Tris HCl [pH 8.3], 1.5 mM MgCl₂, 63 mM KCl, and 1 mM EGTA, 0.1 mg/mL bovine serum albumin, 0.05% Tween 20, 200 μM deoxynucleotide triphosphates, and 15 pmol of MTS primer) at 30°C for 15 minutes. The telomerase-extended products were then amplified by adding 5 μL of amplification reaction mixture (2.5 units of Taq DNA polymerase, 15 pmol of RP-FAM, 0.25 pmol of RPC3g, 0.01 amol of IC, and 7.5 pmol of NT) (Table 2), and PCR was performed in a thermocycler with the following conditions: 3 × (94°C for 30 seconds; 58°C for 60 seconds; and 72°C for 90 seconds) and 25 × (94°C for 30 seconds; 65°C for 30 seconds; and 72°C for 30 seconds). The amplification products were electrophoretically separated in 8% non-denaturing acrylamide gel and visualized with a phosphoimaging system (Typhoon, Molecular Dynamics, Sunnyvale, CA, USA).

Direct effect on telomerase activity in a cell-free system. The indicated concentration of Z. officinale extract was incubated in the telomerase reaction mixture resembling the one for examining the cellular effect on telomerase activity at 30°C for 30 minutes. The crude telomerase (5 ng) was produced from transfected human embryonic kidney 293T cells according to a published method. The subsequent procedures were followed as noted for examining the cellular effect on telomerase activity.

Thin-layer chromatography analysis

Thin-layer chromatography (TLC) fingerprinting of the Z. officinale extract (ethyl acetate fraction) was performed using silica gel GF254 (Fluka, Buchs, Switzerland) as the stationary phase and the solvent mixture of hexane and ethyl acetate (60:40 vol/vol) as the mobile phase. After the

TABLE 2. Oligonucleotides USED IN THE MODIFIED FLUORESCENT TELOMERASE REPEAT AMPLIFICATION PROTOCOL ASSAY

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>MTS</td>
<td>5'-AGCATCCCGTACGAGAGTT-3'</td>
</tr>
<tr>
<td>RPC3g</td>
<td>5'-TAGAGGCACGAGGCGCGTACCGTACGAGAGTT-3'</td>
</tr>
<tr>
<td>RP-FAM</td>
<td>FAM-5'-TAGAGGCACGAGGCGCGTACCGTACGAGAGTT-3'</td>
</tr>
<tr>
<td>IC</td>
<td>5'-TAGAGGCACGAGGCGCGTACCGTACGAGAGTT-3'</td>
</tr>
<tr>
<td>NT</td>
<td>5'-CGATCCCGTACGAGAGTT-3'</td>
</tr>
</tbody>
</table>

Oligonucleotides IC and NT were used as internal controls.
separation, TLC plates were visualized by treating with (A) vanillin–sulfuric acid reagent, (B) vanillin–diethyl ether reagent, (C) vanillin–glacial acetic acid reagent, and (D) anisaldehyde–acetic acid reagent (Supplementary Fig. S2).

High-performance liquid chromatography analysis of 6-gingerol

6-Gingerol was used as a reference compound to quantify the amount of 6-gingerol in the ethyl acetate fraction of *Z. officinale* extract. The high-performance liquid chromatography (HPLC) analyses were performed using an HPLC system (model CTO-10AVP, Shimadzu, Kyoto, Japan) with an ultraviolet-visible spectrophotometric detector (STDM 10AVP, Shimadzu) and a data processor (Class VP 5.0, Shimadzu). The stationary phase was a C18 column (Inertsil ODS-3, 250 mm×4.6 mm, GL Sciences Inc., Tokyo, Japan) with an integrated guard column (Inertsil silica C3-3, 4.6 mm×10.0 mm, GL Sciences Inc.). The mobile phase consisted of acetonitrile and water (70:30 vol/vol), with a flow rate of 1.0 mL/minute. The 6-gingerol was detected at 282 nm with the retention time around 8.1–8.4 minutes. The percentage of 6-gingerol in the *Z. officinale* extract (ethyl acetate fraction) was estimated at 8.9%. A sample of HPLC chromatograms is shown in Supplementary Fig. S3.

Gas chromatography/mass spectrometry

Gas chromatography (GC)/mass spectrometry (MS) data were recorded with a model GC 7890 apparatus from Agilent Technologies (Palo Alto, CA, USA). The gas chromatograph was fitted with a DB-5MS column (30 m 0.25 mm i.d.×0.25 μm film thickness) and used the following temperature programming: 50°C, 5 minutes; to 200°C at 10°C/minute; to 250°C at 5°C/minute; and 250°C, 35 minute; with ionizing voltage of 70 eV and 1 μL split injection (split ratio 25:1). Helium was used as the carrier gas at a flow rate of 1.5 mL/minute.

![Figure 1](image)

**FIG. 1.** Effect of *Z. officinale* extracts on hTERT mRNA expression by semiquantitative reverse transcription-polymerase chain reaction analysis. A549 cells were treated with various concentrations of *Z. officinale* extract from (A) hexane, (B) ethyl acetate, (C) acetone, and (D) methanol for 24 hours. The level of hTERT expression was determined by reverse transcription–polymerase chain reaction analysis. The graphs represent the relative expression of hTERT and its β-variant, after normalization with glyceraldehyde 3-phosphate dehydrogenase expression. The results are expressed as overall mean±SD values from three independent experiments. Statistical significance was determined by Student’s t test (*P<.01 vs. vehicle control).

**RESULTS**

**Growth inhibitory effect of *Z. officinale* extracts in A549 cells**

To establish guidelines for choosing the appropriate range of concentrations in our subsequent experiments, we first determined the growth inhibitory effect of *Z. officinale* extracts in A549 cells using the sulforhodamine B assay. The IC50 values determined for all four fractions were 60±2 μg/mL (hexane), 50±4 μg/mL (ethyl acetate), 60±3 μg/mL (acetone), and >128 μg/mL (methanol). The data represent mean±SD values of three independent experiments.

**Effect of *Z. officinale* extracts on hTERT mRNA expression in A549 cells**

To investigate whether *Z. officinale* extracts down-regulated hTERT at the transcriptional level, A549 cells were treated with *Z. officinale* extract from each fraction for
FIG. 2. Effect of Z. officinale extract (ethyl acetate fraction) on mRNA expression of some telomerase-related genes and the c-Myc gene by semiquantitative reverse transcription–polymerase chain reaction. A549 cells were treated with the ethyl acetate extract of Z. officinale at the indicated concentration for 24 hours. The cDNA was then amplified by polymerase chain reaction using gene-specific primers. The polymerase chain reaction products were visualized by ethidium bromide staining and ultraviolet irradiation. GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

24 hours, and the level of hTERT gene expression was determined by semiquantitative RT–PCR analysis. The graphs in Figure 1 represent the relative expression of hTERT mRNA and its β-variant after normalization with the expression of glyceraldehyde 3-phosphate dehydrogenase mRNA, the internal control gene. The results from Figure 1 show that the Z. officinale extracts from the ethyl acetate and acetone fractions significantly reduced hTERT mRNA expression in a dose-dependent manner, whereas the Z. officinale extracts from the methanol and hexane fractions only slightly decreased hTERT mRNA expression.

Effect of Z. officinale extracts (ethyl acetate fraction) on expression of telomerase-related genes and c-Myc

Because the ethyl acetate fraction decreased hTERT mRNA expression the most, we focused on further investigations on this fraction. Figure 2 shows the semiquantitative RT–PCR results of the A549 cells treated with Z. officinale extract (ethyl acetate fraction) for 24 hours. These results demonstrate that the extract significantly reduced hTERT mRNA and c-Myc mRNA in a concentration-dependent manner, whereas the other telomerase-related genes (human telomeric RNA, TRF1, TRF2, and human TEPI) and glyceraldehyde 3-phosphate dehydrogenase were not affected.

We further confirmed that the down-regulation of hTERT and c-Myc gene expression by Z. officinale extract led to the reduction of hTERT and c-Myc at the protein level. Using western blotting analysis, Figure 3A shows that levels of hTERT and c-Myc from the A549 cells treated with Z. officinale extract (ethyl acetate fraction) for 48 hours were reduced in a concentration-dependent manner. Moreover,

**FIG. 3. Effect of Z. officinale extract (ethyl acetate fraction) on hTERT and c-Myc expression by western blotting analysis. (A) A549 cells were treated with the indicated concentration of the ethyl acetate extract of Z. officinale for 48 hours before analysis by western blotting. Lane T is the sample from hTERT-transfected A549 cells and thus serves as the hTERT indicator. (B) A549 cells were treated with the extract at 40 µg/mL for the indicated time before the analysis.**

2B shows that levels of both hTERT and c-Myc from the A549 cells treated with Z. officinale extract (ethyl acetate fraction) at 40 µg/mL were reduced in a time-dependent manner.

Effect of Z. officinale extracts (ethyl acetate fraction) on telomerase activity

We further investigated the telomerase activity of the treated A549 cells using a modified TRAP assay. A549 cells were treated with the extract for 24 hours before the cells were lysed, and the crude cellular proteins were used as crude telomerase extract. Figure 4A shows that telomerase

**FIG. 4. Effect of Z. officinale extract (ethyl acetate fraction) on telomerase activity. (A) The cellular effect on telomerase activity. A549 cells were treated with the indicated concentration of the ethyl acetate extract of Z. officinale for 24 hours before 200 ng of the crude cell lysate was used as the source of telomerase in a modified fluorescent telomerase repeat amplification protocol assay. (B) The direct effect on telomerase activity in a cell-free system. The indicated concentration of the extract was directly incubated in the modified fluorescent telomerase repeat amplification protocol assay reaction mixture. IC represents the internal control. Lane C represents the negative control experiment when telomerase was heat-denatured.
activity in the cells treated with the extract was reduced in a concentration-dependent manner.

We questioned whether telomerase inhibition could have arisen from the direct inhibition of the enzyme itself. Therefore, we tested Z. officinale extract (ethyl acetate fraction) directly in a cell-free system TRAP assay. Figure 4B shows that even with the extract at 250 μg/mL (a concentration much higher than the one used with the A549 cells), telomerase activity was still not inhibited.

All of these experiments show convincingly that Z. officinale extract, especially from the ethyl acetate fraction, can down-regulate hTERT expression, leading to a reduction in telomerase activity for the treated cells. The reduced telomerase activity is likely due to diminished protein production rather than direct inhibition of the enzyme. This down-regulation of hTERT expression paralleled the down-regulation of c-Myc. Since c-Myc and Max form heterodimers that activate hTERT expression, it is likely that the down-regulation of c-Myc precedes the down-regulation of hTERT.

Analyses of Z. officinale extract (ethyl acetate fraction) by TLC, HPLC, and GC/MS

In an effort to identify the active compound that is responsible for the down-regulation of hTERT and c-Myc, we used TLC, HPLC, and GC/MS to examine the composition of the ethyl acetate fraction of Z. officinale extract. The TLC and HPLC fingerprints (Supplementary Figs. S2 and S3) show that 6-gingerol is the major compound found in the fraction, which is estimated at about 8.9% of the extract. The GC chromatogram showed 24 peaks between the retention times of 18 to 50 minutes (Supplementary Fig. S4). Based on reference data from the mass spectral library, the peaks with retention times between 18 and 20 minutes were identified as ar-curcumin (18.49 minutes), zingeriberene (18.66 minutes), ć-farnesene (18.71 minutes), β-bisabolene (18.83 minutes), and sesquiphellandrene (19.04 minutes). The majority of the remaining peaks eluted from 20 to 40 minutes contained the base peak of m/z 137, which is the predominant mass spectral feature of gingersols, paradols and shogaols, but not dehydrogigertoines. Based on the fragment analysis of compounds in ginger reported by Jolad et al., we found zingeriberene (20.46 minutes), 6-shogaol (29.54 minutes), 6-dehydro-6-gingerol (30.04 minutes), 6-gingerol (31.13 minutes), 8-shogaol (33.11 minutes), 10-shogaol (31.71 minutes), and 11-paradol (40.01 minutes), among others (a few chromatograms are shown in Supplementary Fig. S5). Notably, the peak areas in the GC chromatogram do not correlate directly with the amounts of material present because the chromatogram was generated from the total ion current as a function of time. Furthermore, the thermal degradation and dehydration of gingersols under gas chromatographic conditions produces aliphatic aldehydes, zingeriberene, and the corresponding shogaol.

Because 6-gingerol represented the most prominent peak in our extract, we purchased pure 6-gingerol from two sources (Sigma and China Aroma Chemical) to test whether this compound might be responsible for the observed down-regulatory effect on hTERT and c-Myc expression. However, based on RT–PCR analysis, the A549 cells treated with the compound from both sources showed no significant reduction in hTERT and c-Myc expression (Supplementary Fig. S6). Therefore, the compound that is responsible for the down-regulation of hTERT and c-Myc is not 6-gingerol, but might be one of the other compounds identified above, or the synergistic effect of two or more compounds, or a different compound that is not readily detected by GC/MS.

DISCUSSION

Various compounds in ginger (Z. officinale Roscoe) have been reported to have anticancer properties in a wide range of experimental models. For example, 6-gingerol suppresses colon cancer growth by targeting leukotriene A4 hydrolase,27 stimulates apoptosis through up-regulation of NAG-1 and cell cycle arrest by down-regulation of cyclin D1 in human colorectal cancer cells,28 inhibits cell adhesion, invasion, and motility as well as the activities of matrix metalloproteinase-2 and -9 in MDA-MB-231 human breast cancer cell lines,29 and inhibits angiogenesis,30 among other activities.31 6-Shogaol induces autophagy by inhibiting the AKT/mTOR pathway in A549 human lung cancer cells,31 inhibits proliferation of the transgenic mouse ovarian cancer cell lines,32 and induces apoptosis in human colorectal carcinoma cells via reactive oxygen species production, caspase activation, and GADD153 expression.33 6-Paradol decreases the incidence and the multiplicity of skin tumors initiated by 7,12-dimethylbenz(a)anthracene and promoted by 12-O-tetradecanoylphorbol 13-acetate,34 exerts inhibitory effects on the viability and DNA synthesis of human promyelocytic leukemia (HL-60) cells,35 and induces apoptosis in an oral squamous carcinoma cell line.36 In the present study, we found that the ethyl acetate fraction of ginger rhizome extract down-regulates hTERT and c-Myc expression in A549 human lung cancer cells in a dose- and time-dependent manner. The down-regulation of hTERT consequently led to a decrease in telomerase activity in these cells. Our findings add two more cancer-related molecular targets to the list of targets highlighted here.

The observation that telomerase is active in cancer cells but not in normal somatic cells has generated a great deal of interest in the development of telomerase inhibitors for use as anticancer agents. Telomerase inhibition is probably most useful in chemoprevention, the early stages of carcinogenesis, or maintenance therapy after treatment by cytotoxic chemotherapy because of its specificity and delayed response in cancer cells.9 Recent studies suggest that cancer stem cells already possess some telomerase activity; therefore, rather than telomerase reactivation, enzyme activity might increase in the terminal stages of carcinogenesis because of an increased expression or efficient assembly of telomerase components.37 Because the ginger extract used in this study down-regulates hTERT and reduces telomerase
activity, it should prove useful in cancer prevention and maintenance therapy.

There are at least 430 genes that have been found to possess a Myc-bound promoter; 13% of these genes are related to protein synthesis, and 12% are related to cell cycle proteins. Myc increases cell size by means of an increase in global protein synthesis. It is speculated that the increase in cell growth might promote cell proliferation downstream of Myc activation, in addition to Myc’s ability to regulate key cell cycle proteins. Although Myc, in principle, should be an attractive target for cancer therapy, the lack of direct evidence and the concern about the safety issues have hindered the development of Myc inhibitors as anticancer agents. Recently, there is renewed interest in Myc inhibition for cancer treatment. The experiments in a conditioned-mouse model showed that Myc inhibition triggered rapid regression of a Ras-dependent tumor, with well tolerated and reversible side effects, normal regenerating tissues. These experiments demonstrate the feasibility of Myc inhibition as an effective and efficient cancer therapy. Because our ginger extract can clearly down-regulate c-Myc in a dose- and time-dependent manner, our results indicate that ginger extract might prove to be an effective chemopreventive anticancer agent.

Taken as a whole, the results from this study reveal that the ethanolic fraction of ginger, Z. officinale, rhizome extract down-regulates hTERT and c-Myc expression in A549 human non-small cell lung cancer. The down-regulation of hTERT leads to a reduction in both protein production and telomerase activity. The down-regulation of hTERT might follow the down-regulation of c-Myc because c-Myc regulates hTERT transcription. As both telomerase inhibition and Myc inhibition are cancer-specific targets for cancer therapy, ginger extract might prove to be beneficial as a complementary agent in cancer prevention and maintenance therapy.

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AUTHOR DISCLOSURE STATEMENT

No competing financial interests exist. All the authors report no conflict of interest and accept full responsibility of the article’s content.

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