Blocking Telomerase by Dietary Polyphenols Is a Major Mechanism for Limiting the Growth of Human Cancer Cells *in Vitro* and *in Vivo*[1]

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ABSTRACT

Animal and epidemiological studies reveal that consuming food and beverages rich in polyphenols (*e.g.,* catechins, flavones, and antocyanines) is associated with a lower incidence of cancer, and several molecular mechanisms have been proposed for explaining this effect. However, because most of these mechanisms were observed only under specific and nonphysiological conditions, and in most cases, with practically irrelevant concentrations, there is still no clear-cut or universal explanation for the major events that underlie the anticancer effects of polyphenols. In this study we present clear *in vitro* and *in vivo* evidence that the inhibition of the cancer-associated enzyme telomerase is a key mechanism involved in cancer inhibition by epigallocatechin gallate (EGCG), a major tea polyphenol. We demonstrate that EGCG and other selected polyphenols undergo structural rearrangements at physiologically permissible conditions that result in remarkably increased telomerase inhibition. In nude mice models bearing both telomerase-dependent and -independent xenograft tumors cloned from a single human cancer progeny, only the telomerase-dependent tumors responded to prolonged oral administration of EGCG. Thus, EGCG and likely other structurally related dietary polyphenols seem to act as prodrug-like molecules that, once ingested and distributed, undergo structural changes that favor potent activity against telomerase.

INTRODUCTION

Dietary polyphenols are ubiquitous groups of plant metabolites that commonly occur in the human diet (fruits, vegetables, and beverages). One of the major groups of dietary polyphenols is the flavonoids, which mainly comprise the catechins and the flavonols (*e.g.,* quercetin and myricetin; reviewed in Ref. 1). In recent years, several lines of evidence from epidemiological and animal studies have emerged, showing chemopreventive and anticancer potential of dietary polyphenols (1–7). For example, several studies have suggested positive correlations between human consumption of green tea and a lower incidence of gastric, esophageal, ovarian, pancreatic, and colorectal cancers (8–11). Furthermore, EGCG,[3] the major polyphenol in green tea, was found to effectively and broadly inhibit carcinogenesis in various animal organs such as the esophagus, stomach, duodenum,

colon, liver, pancreas, lung, breast and skin (reviewed in Ref. 12). Various mechanisms were suggested to explain the chemopreventive and anticancer effects of polyphenols. Antioxidant activities, and interaction with certain enzymes or proteins implicated in cancer (*e.g.,* urokinase, ornithine decarboxylase, NADPH-cytochrome P450 reductase, protein kinase C, steroid 5 α reductase, tumor necrosis factor and epidermal growth factor expression, nitric oxide synthase, and cyclooxygenase 2) were postulated. However, because many of these mechanisms were observed only with specific targets, or were examined under exceptional and nonphysiological conditions, and in most cases, with practically irrelevant concentrations, it is still not clear how polyphenols uniquely affect cancer in a universal, selective, and nontoxic process.

In an earlier *in vitro* study we have reported that inhibiting the cancer-associated enzyme telomerase by tea polyphenols may provide a plausible mechanism for explaining the anticancer effects of these molecules (13). Telomerase, a specialized reverse transcriptase, has a salient role in the process of immortalization and tumorigenesis. During cell cycle progression of a normal cell, DNA polymerase completely duplicates the genomic DNA except on the very ends of the chromosomes, the telomeres (reviewed in Ref. 14). As a consequence, the telomeric end shortens an average of 50–200 bp each time the cell divides. Because of the problem of telomere shortening, normal diploid cells are "mortal" and have limited capacity to proliferate. However, immortalized cells overcome the obstacle of telomere shortening by evolving special mechanisms for telomere maintenance. In most tumors, the maintenance of telomeres is achieved through the expression of telomerase, which stabilizes and elongates telomeres by the *de novo* synthesis of telomeric DNA. The role of telomerase in immortalization was confirmed recently by the findings that the ectopic expression of telomerase in various normal cells resulted in the extension of the life span of these cells (15, 16). Telomerase activity has been identified in most human tumors but is absent in the majority of somatic cells (17). High telomerase activity correlates with the degree of malignancy and the likelihood of tumor progression (18, 19). Furthermore, there is accumulating evidence that telomerase functions as a DNA repair and antiapoptic enzyme in addition to its role in telomere maintenance (20–23). A recent report showed that *in vitro* antitelomerase treatment of breast epithelial cells from women with Li-Fraumeni syndrome significantly decreased the frequency of spontaneous immortalization of these preimmortal cells (24). Together these findings validate the view that inhibition of telomerase function may constitute a new strategy of chemoprevention and antineoplastic therapy.

In conjunction with our previous study on the inhibition of telomerase by tea catechins (13), the present study additionally demonstrates that EGCG and some selected dietary polyphenols (epicatechin, quercetin, myricetin, naringin, naringinin, and biochanin A) undergo, at neutral or alkaline pHs, structural degradation that results in remarkably increased telomerase inhibition. Using both *in vitro* cell cultures and *in vivo* transplanted tumor models established with telomerase-dependent and -independent cancer cells confirmed the te-
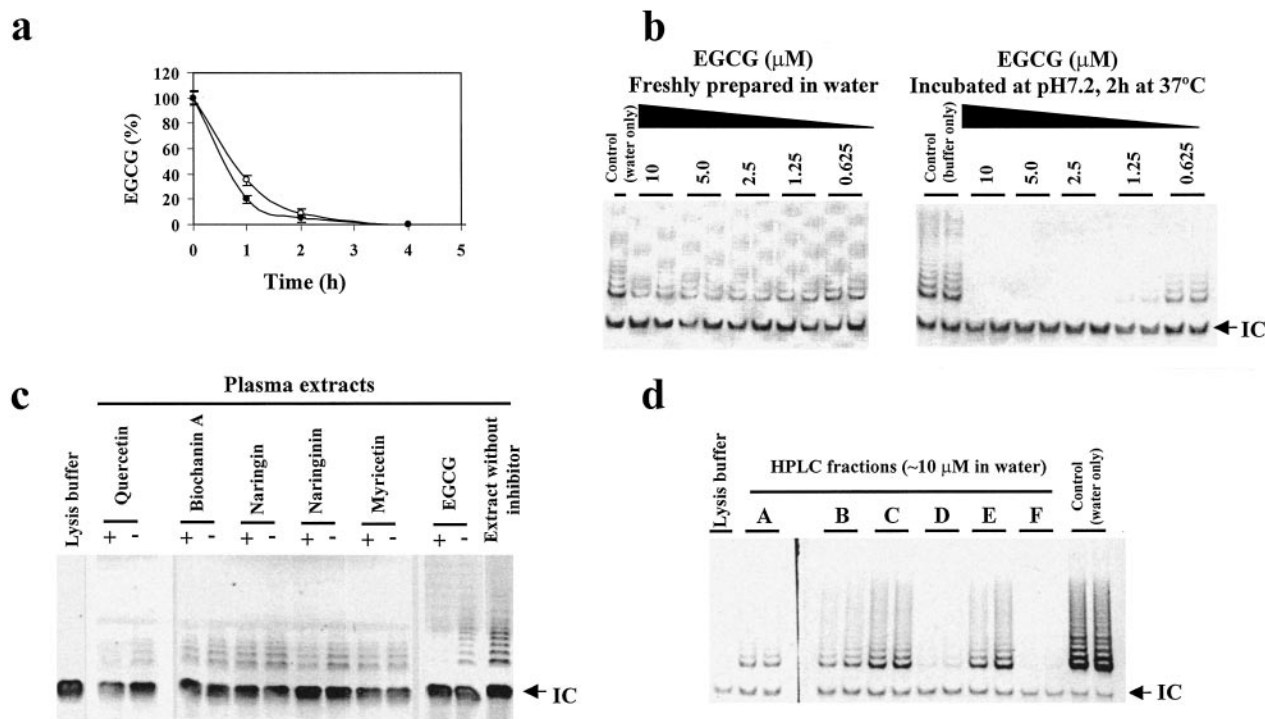
Fig. 1. *In vitro* cell-free inhibitory effects of polyphenols on telomerase activity. *a*, stability of EGCG in cell culture medium (RPMI 1640 + 5% FBS; *open symbols*) or human plasma (*closed symbols*). *b*, concentration dependency of telomerase inhibition by fresh or buffer-incubated EGCG [Tris-HCl (pH 7.2)]. *c*, effect of incubating various polyphenols (10 μM each) in human plasma on their potency of inhibiting telomerase. *d*, inhibitory effects of several HPLC fractions of the incubated EGCG sample from *b* (HPLC aliquots corresponding to each fraction were evaporated to remove organic solvent, and then were reconstituted in distilled water and tested. The final molar concentrations were calculated as equivalents to the concentration of the original EGCG before incubation). Plasma extraction was performed immediately (−) or after 2 h incubation at 37°C (+). *IC*, PCR internal control; *bars*, ±SD.

lomerase inhibition-mediated effect of EGCG (as a representative polyphenol) on cancer.

MATERIALS AND METHODS

Reagents and Cell Lines. EGCG and other polyphenols were purchased from Sigma Chemical Co. (St. Louis, MO). U937 lymphoblastic leukemia cells, HCT-116 colon carcinoma cells, and Saos-2 osteosarcoma cells were purchased from American Type Culture Collection (Rockville, MD). HTERT-RPE1 cells were purchased from Clontech (Palo Alto, CA). Human normal foreskin fibroblasts were purchased from Sanko (Tokyo, Japan). All of the cell lines were cultured according to the manufacturer's instructions. Unless otherwise mentioned, distilled water was the vehicle used to dissolve all of the compounds tested.

TRAP and TRF Assays. TRAP assay was performed essentially as described previously (17, 25), with a set of primers (TS, 5′-AATCCGTCGAG-CAGAGTT-3′; ACX, 5′-GCGCGGCTTACCCTTACCCTTACCCTAACC-3′; NT, 5′-ATCGCTTCTCGGCCTTTT-3′) and an internal standard, TSNT (5′-AATCCGTCGAGCAGAGTTAAAAGGCCGAGAAGCGAT-3′). The telomeric products were separated by PAGE and visualized by staining with SYBR Green (Takara, Kyoto, Japan). On the basis of testing serial dilutions of cell lysates (10–10,000 cells/reaction), an amount of lysate equivalent to 1,000 cells/reaction was found to be within the maximum quantitative capacity of the TRAP assay and was adopted for all of the cell types tested. The effect of incubation in plasma on telomerase inhibitory effect of polyphenols was performed on plasma extracts prepared according to procedures reported previously (26, 27), and 10 μl of the finally reconstituted solution was used in the TRAP assay. TRF assay was performed by standard Southern blotting procedures using the TeloQuant assay kit (PharMingen) as described previously (25).

TUNEL, BrdUrd Incorporation, and SA-β-Gal. Apoptosis in tumor cells and tissues was determined by the TUNEL technique, using the TACS 2 terminal deoxynucleotidyltransferase kit (Trevigen). BrdUrd incorporation was

performed using the cell proliferation assay kit (Roche), and SA-β-gal activity was determined according to Dimri *et al.* (28).

FISH. Chromosomal spreads were prepared according to standard procedures using 1-h treatment with colcemide (0.1 μg/ml). Telomere visualization was performed by using a Cy3-conjugated PNA probe according to the manufacturer's instructions (DAKO). For the detection of telomeres in tissue sections, the labeling procedures were modified as follows: 10-μm cryosections mounted on glass slides were fixed with 3.7% formaldehyde at room temperature for 15 min. The slides were then washed with PBS and treated with Cytopore (Trevigen) for 20 min at room temperature. After brief washes with Tris-buffered saline, the experiment was pursued then using the Cy3-conjugated PNA telomere labeling kit exactly as instructed by the manufacturer. Quantification of captured images was performed using NIH ImageJ/Adobe Photoshop. Values of telomeric spot area (Cy3 signals) divided by total nuclear area (DAPI signals) were compared between treated and nontreated groups at equal exposure times. Approximately 40 microscopic fields (~50 nuclei/field) in nonapoptotic areas were examined in each treatment group. Calibration of the method was established using tissue sections with known telomeric length as determined by Southern blot. The functionality of DAPI in the quantitation of images was confirmed by capturing images at three expo-

Table 1 *Effect of neutral buffer treatment [Tris-HCl (pH 7.2)] on the IC_{50} of various plant polyphenols[a]*

Compound	With incubation	Without incubation
EGCG	0.3	8.5
Quercetin	0.45	4.9
Myricetin	nd[b]	2.1
Ellagic acid	nd	0.39
Pyrogallol red	nd	0.47
Epicatechin	0.3	>10.0
Gallic acid	>10.0	>10.0

[a] IC_{50} values were determined by duplicate measurements at four different concentration points.
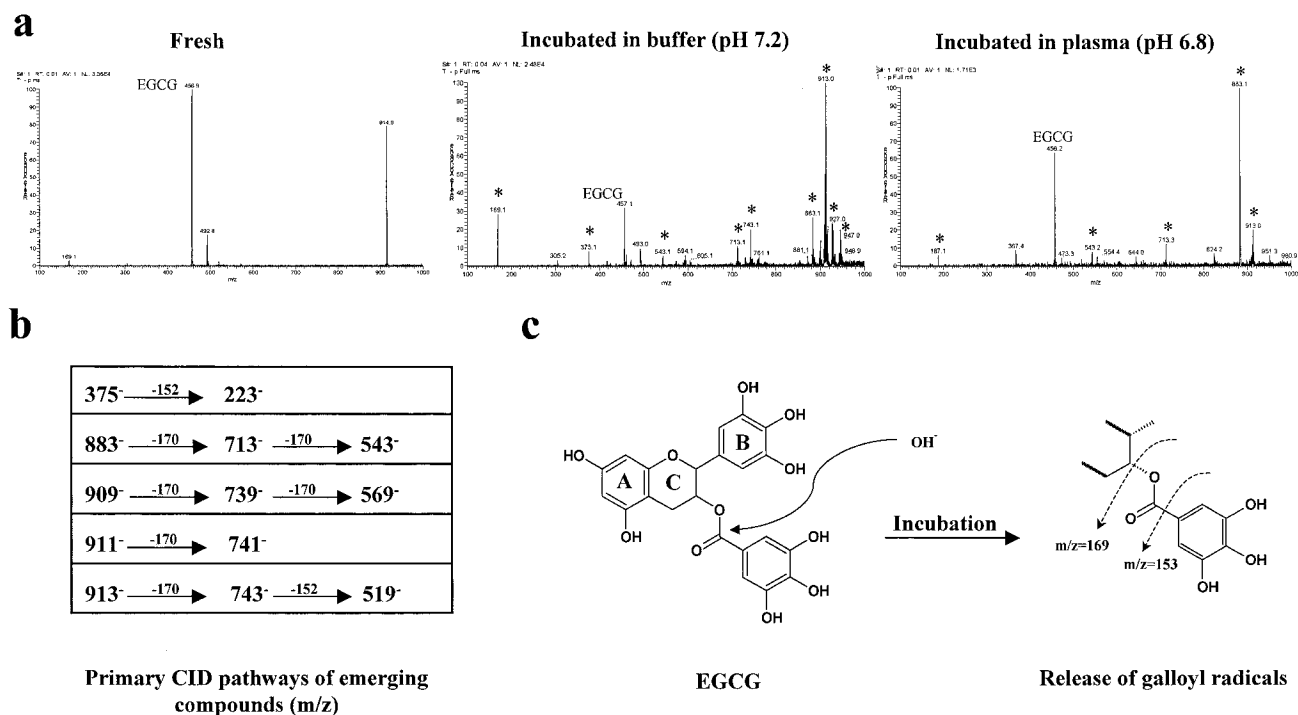
[b] nd, not determined.

Fig. 2. *a,* mass spectrometry analysis of EGCG degradation after incubation in pH 7.2 buffer or human plasma. ∗, common degradation species found after incubation in both plasma and buffer as represented by *m/z* values. *b,* primary CID pathways as determined from MS/MS spectra of major peaks found in incubated EGCG samples. *c,* EGCG chemical structure and the speculated release of galloyl radicals on incubation.

sure times (3, 7, and 9 s), where the ratios of Cy3 signal:DAPI signal were always comparable among the three sets of exposure, indicating that at these exposure times both Cy3 and DAPI signals were below saturation.

Transfection. A pOPRSVI/MCS vector (Stratagene) encoding the 185-bp flanking region of the hTR (29) was kindly provided by Dr. Yiqun Mo (Ohio State University, College of Pharmacy). Stable transfection was performed using the Effectene kit (Qiagen) and the selection agent G418 (500 μg/ml). After selection, each clone was divided into four flasks for propagation.

Degradation, HPLC, and Mass Spectrometry. Freshly prepared EGCG solutions (typically 20–200 μM) in plasma, culture medium or buffer [20 mM Tris-HCl or PBS (pH 7.2)] were used for the incubation and degradation study. Buffer incubated samples were analyzed directly; however, plasma and medium incubated samples were extracted according to procedures reported previously (26, 27) and then analyzed. In a typical experiment, approximately 10–50 μl of ~50 μM EGCG solution/extract in mobile phase was injected into an HPLC system (Shimadzu LC-6A) equipped with a UV detector (Shimadzu SPD-6AV) set at 220 nm and with a C18 column. The mobile phase consisted of 20% methanol in 10 mM acetic acid (pH 3.0). HPLC-MS and MS-MS were performed using an ESI LCQ ion trap mass spectrometer (Finnigan) tuned at negative mode with helium as the collision gas and at an electrospray-mass spectrometry voltage of 6 kV. Typically, ~100 μl of fresh or degraded EGCG solution (10 μM) was used in each run of analysis.

In Vivo **Treatment.** BALB/c nude female mice were purchased from Charles River Laboratories (Yokohama, Japan) and maintained according to institutional regulations. HCT-L2 and HCT-S2R cells (5 × 10⁶ cells in 0.1 ml of serum-free DMEM) were inoculated s.c. into the right flank of 8-week-old mice (6 mice for each treatment group). Tumor growth was monitored using calipers every 2 or 3 days. Tumor size was calculated as (L+W)/2, where L = length and W = width. Six days after inoculation, drinking water bottles were replaced by EGCG or vehicle solutions. EGCG powder (Sigma) was dissolved in vehicle [0.01% ascorbic acid solutions (pH 5.5) adjusted by NaOH]. Administered solutions were replaced every other day, and the stability of EGCG in the vehicle system was confirmed by HPLC-UV to be >90% over a 48-h period. On the final day of treatment, mice were sacrificed, tumors were removed and frozen, and 10-μm cryosections were prepared for histological studies.

RESULTS

Degraded Dietary Polyphenols Are Potent Telomerase Inhibitors. The fate of polyphenols in living systems is still not well defined, and the few bioavailability studies available thus far showed that most types of polyphenols have limited bioavailability and apparently undergo extensive metabolism after ingestion (30). As we reported in our previous study (13), EGCG and other related polyphenols were found to be effective telomerase inhibitors. However, we noticed during additional investigations on the inhibitory effect of EGCG on telomerase activity that briefly incubating EGCG in neutral or slightly alkaline medium resulted in rapid chemical degradation of parent EGCG accompanied by a dramatic enhancement in the telomerase inhibition (~20-fold; Fig. 1, *a* and *b*). Similarly, we found that a brief incubation of EGCG in human or mouse plasma (pH 6.8) at 37°C also resulted in rapid degradation and increased inhibitory activity. Interestingly, the increase in telomerase inhibitory activity after incubation was also observed with other major plant polyphenols tested (Fig. 1*c*; Table 1). HPLC-MS analyses showed that EGCG and likely other related polyphenols undergo a series of complex structural changes that include auto-oxidation, oxidative addition, and/or condensation, leading to a variety of new structures (Fig. 1*d,* and Fig. 2a).[4] Analysis with CID suggested the involvement of the galloyl group in the various addition/condensation *reactions (Fig. 2, b* and *c).* The complexity and the additive nature of these structures are in agreement with recent studies (27, 31–33), although the majority of these studies naively reasoned that the near absence of parent polyphenols in the blood stream was because of extensive metabolism. A recent study on the oxidation of EGCG with peroxyl radicals generated by thermolysis of the initiator 2,2′-azobis(2,4-dimethylvaleronitrile) referred to the formation of dimers and B-ring opened oxidation

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[4] Unpublished observations.

products (34). However, the molecular weights of the oxidation products mentioned in the above study were not consistent with the molecular weights of the degradation products in the present study, indicating different reaction pathways. Additional analyses are needed to establish the exact nature of the chemical structures emerging from the degradation of EGCG and other polyphenols. Nevertheless, it is obvious that chemical instability plays a major role in determining the fate of polyphenols *in vivo*.

The Long-Term Effect of EGCG Is Exclusive to Telomerase-dependent Cells. To establish the specificity of EGCG effects on telomerase in cell cultures, we examined its long-term effect using two normal cell lines with negative and positive telomerase activity, and two immortal cell lines also with negative and positive telomerase activity. EGCG at 5 μM (added to culture medium once every 4 days) induced marked shortening of telomeres that led to cellular senescence and death in U937 leukemia cells, and in the telomerase-immortalized normal retinal epithelial cells (hTERT-RPE1; Clontech). However, it did not induce telomere shortening or premature cell death when tested at 10 μM on the telomerase-negative Saos-2 osteosarcoma cells or normal human foreskin fibroblasts (Fig. 3, *a* and *b*). To additionally substantiate the linkage between EGCG long-term toxicity and telomerase inhibition, and to exclude the possibility that the effect of EGCG is cell-type dependent, we then tested telomerase inhibition by EGCG on cell lines derived from a single parent population that expressed different telomere lengths. To this end we subcloned HCT-116 human colon carcinoma cells by the limiting dilution method to obtain sublines with various telomeric lengths from 1.5 to 7.0 kb (estimated as the TRF). All of the sublines showed similar levels of telomerase activity, and the mean TRF of each subline remained stable throughout prolonged periods of cultivation ($\sim$4 months; data not shown). Two sublines with short (1.5 kb, designated as HCT-S1) and long telomeres (7.0 kb, designated as HCT-L1) were subjected to prolonged EGCG exposure at 5 and 10 μM. As expected, HCT-S1 cells demonstrated changes associated with cellular senescence and death at an earlier time (PDs <20) than the HCT-L1 cells which entered crisis at PDs >30 (Fig. 3, *c–e*). It is noteworthy that the cellular toxicity observed in HCT-S1 cells appeared to occur mainly through growth arrest and/or senescence, whereas, for a yet-unidentified reason, the cellular toxicity observed in HCT-L1 cells appeared to progress through an apoptotic pathway. Interestingly, we found that one subline with an average telomere length of 2.0 kb was strikingly resistant to the prolonged effect of EGCG, despite showing unchanged acute sensitivity (estimated as IC_{50} values) toward EGCG itself as well as various standard cell poisons (Table 2). This subline (designated hereafter as HCT-S2R) exhibited common resistance to prolonged cultivation with other known telomerase inhibitors FJ5002 (at 50 nM) and AZT (at 5.0 μM; Fig. 4a). To directly confirm that HCT-S2R cells were specifically
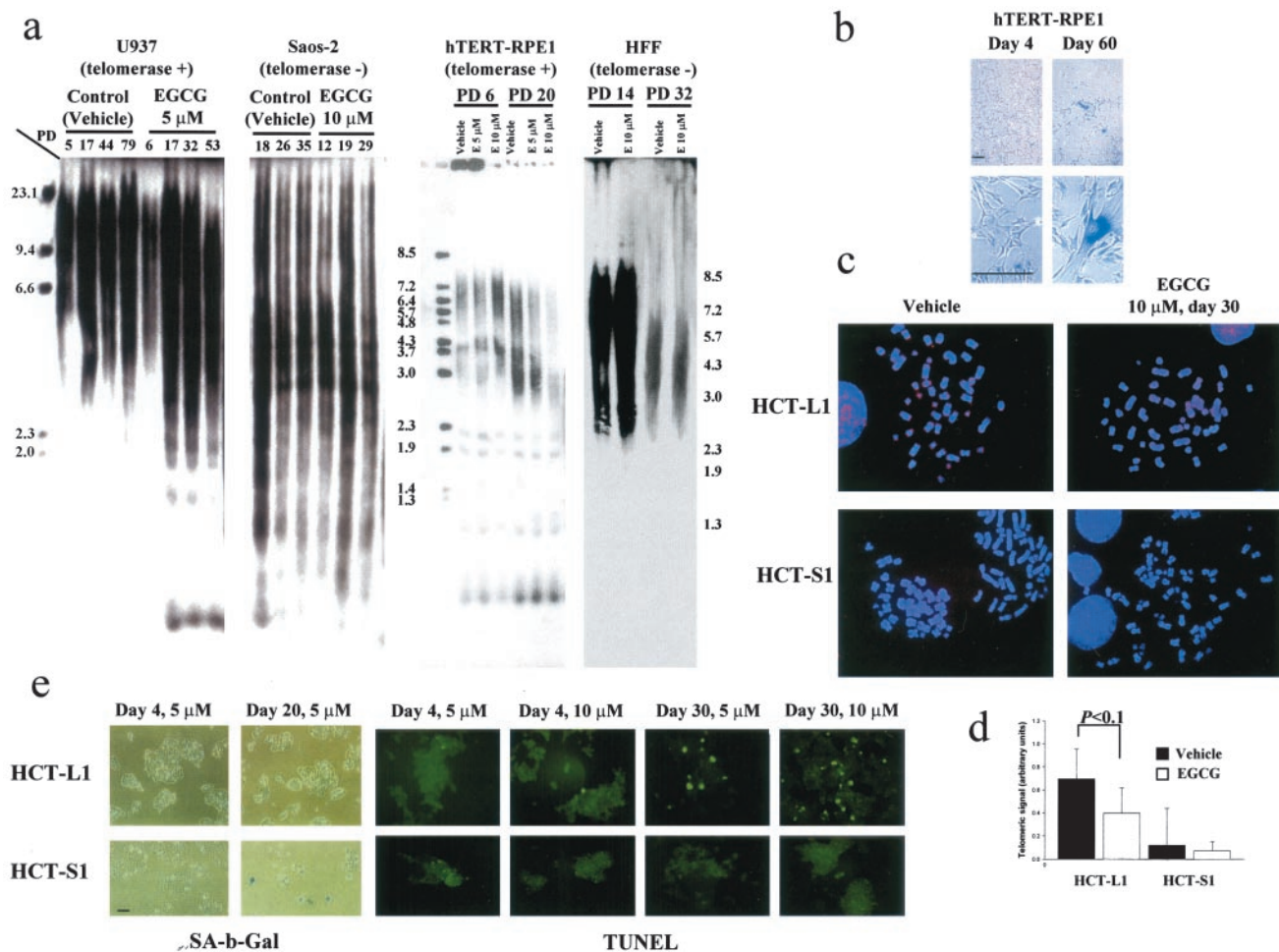
Fig. 3. Cellular effects of the prolonged cultivation of cancer (U937, Saos-2, HCT-L1, and HCT-S1) and normal (hTERT-RPE1 and HFF) cells with subtoxic concentrations of EGCG. *a–c*, telomere shortening and late cellular toxicity after treatment with EGCG is observed only in the telomerase-positive U937, hTERT-RPE1, and HCT-116 cells (HCT-L1 and HCT-S1 clones). *d*, quantitation of the telomeric signals in FISH analysis (25 spreads for each treatment); *bars*, ±SD. *e* (and *b*), morphology changes and SA-β-gal activity in HCT-S1, HCT-L1, and hTERT-RPE1 cells; and wide-spread apoptosis (TUNEL) in HCT-L1 cells at day 30 of cultivation with EGCG. Cells were passaged every 4 days with the addition of fresh EGCG at the indicated concentrations. *Bar* = 50 μm.

Table 2 IC_{50} *of several cellular poisons tested on HCT-L2 and HCT-S2R cells*[a]

Compound	HCT-L2	HCT-S2R
Vincristine	4.0 nM	3.8 nM
Paclitaxel	20.6 nM	17.4 nM
Camptothecin	18.0 nM	17.0 nM
G418	280 mM	285 mM
EGCG	40 mM	35 mM

[a] IC_{50} values were determined by interpolation from the concentration-growth response curves of duplicate measurements.

resistant to telomerase inhibition, we stably introduced into these cells a plasmid vector that generates antisense against the template region of the telomerase RNA subunit (hTR). As a positive control we also introduced the antisense hTR plasmid into a counterpart subline (designated hereafter as HCT-L2 cells; TRF ~6.6). Clones with maximal telomerase inhibition, as determined by TRAP assay, were selected for propagation (Fig. 4b; clones number 4 from both cell lines). Initially, all of the selected HCT-S2R and HCT-L2 clones containing antisense or empty vectors grew at similar rates. However, from 25 to 30 PDs post-transfection, only HCT-L2 clones containing antisense vector went into senescence and crisis as judged from morphology, acidic β-galactosidase activity, and rate of BrdUrd incorporation (Fig. 4, c and d). All of the other clones continued to grow normally throughout the course of the experiment (~2 months) despite the continued telomerase inhibition of the antisense hTR-transfected HCT-S2R cells as confirmed by TRAP assay. At this stage it is

unclear how these cells survive telomerase inhibition. However, it seems that because these cells have remarkably short telomeres, they have evolved auxiliary mechanisms to prevent telomere erosion in the absence of telomerase activity. Nevertheless, it is clear from the above findings that the resistance of these cells is exclusive to telomerase inhibitors but not other cytotoxic agents.

Effect of the Oral Administration of EGCG on the Growth of Human Tumors in Nude Mice. Because most telomerase-negative cancer cell lines lack the ability to induce tumors in nude mice,[4] we elected to take advantage of HCT-S2R cells and to use them as a negative control to determine the selectivity of EGCG against telomerase *in vivo*. EGCG solution (0.04%) was p.o. administered as the sole source of water to nude mice bearing tumors established from either HCT-L2 cells or HCT-S2R cells. Preliminary experiments showed that both sublines induce tumors in nude mice at equal rates, as it is the case of the original parent cell line (data not shown; American Type Culture Collection information sheet). The average water consumption was constant throughout the experiment and was estimated at 2.85 ± 0.4 ml/mouse/day (equivalent to 1.14 ± 0.16 mg/mouse/day of EGCG). As shown in Fig. 5a, initially all of the tumor groups grew at equal rates. However, beginning from approximately day 50, only the EGCG-treated group of HCT-L2 tumors started to diminish in size, all of the animals appeared healthy with no loss of body weight, and by the end of the experiment 2 of 6 mice showed complete remission (average body weight 22.6 ± 1.5 g).
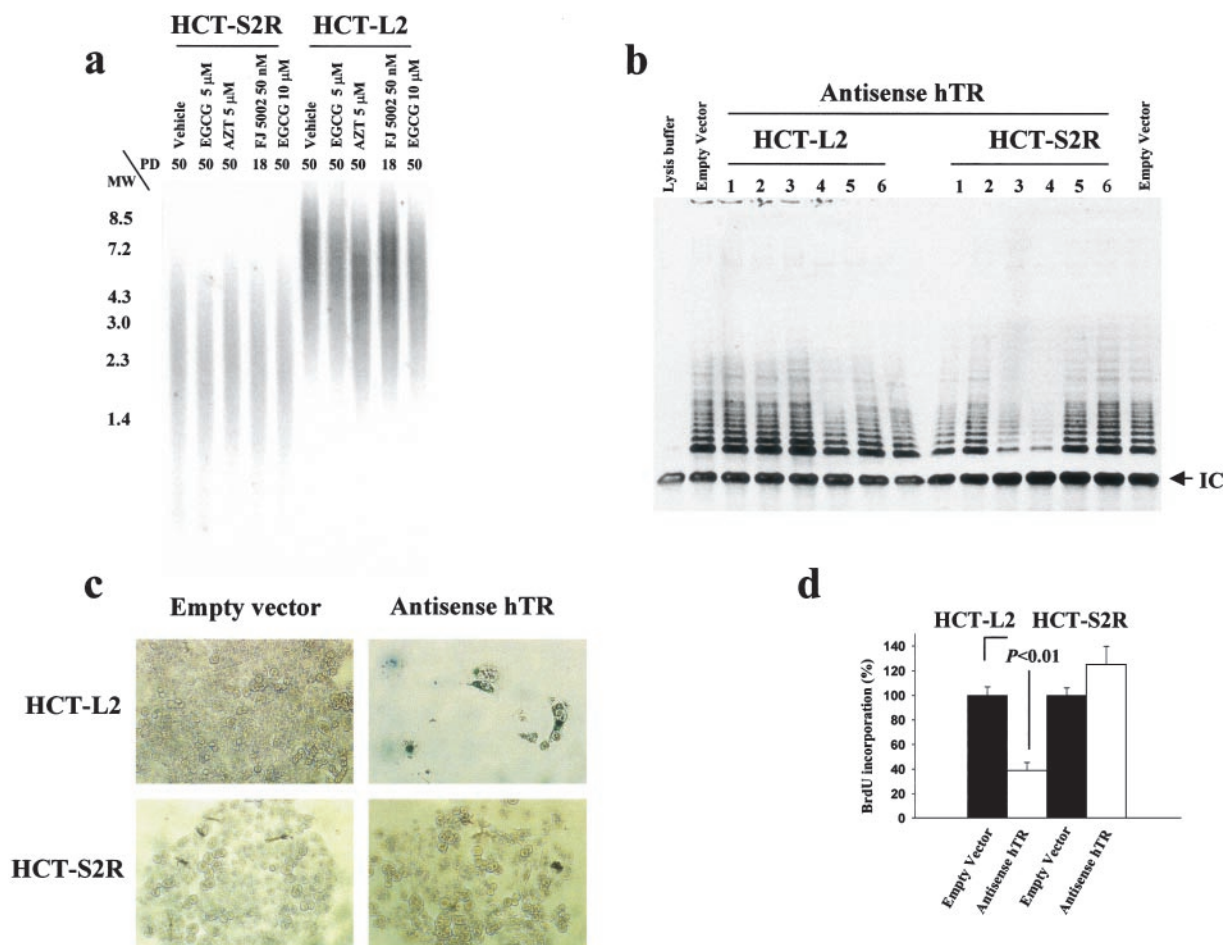

Fig. 4. Specific resistance of HCT-S2R cells to telomerase inhibition. *a,* telomerase inhibitors induced telomere shortening in HCT-L2 cells but not in HCT-S2R cells as determined by TRF measurement. *b,* telomerase activity in clones transfected with empty or antisense hTR vector. *c,* morphology changes and senescence of HCT-L2 cells after ~30 days of transfection. *d,* senescence of HCT-L2 cells but not HCT-S2R cells as determined by the ratio of BrdUrd uptake after 12 h. At approximately day 30 both cell lines continued to have decreased telomerase activity (data not shown); *bars,* ±SD.
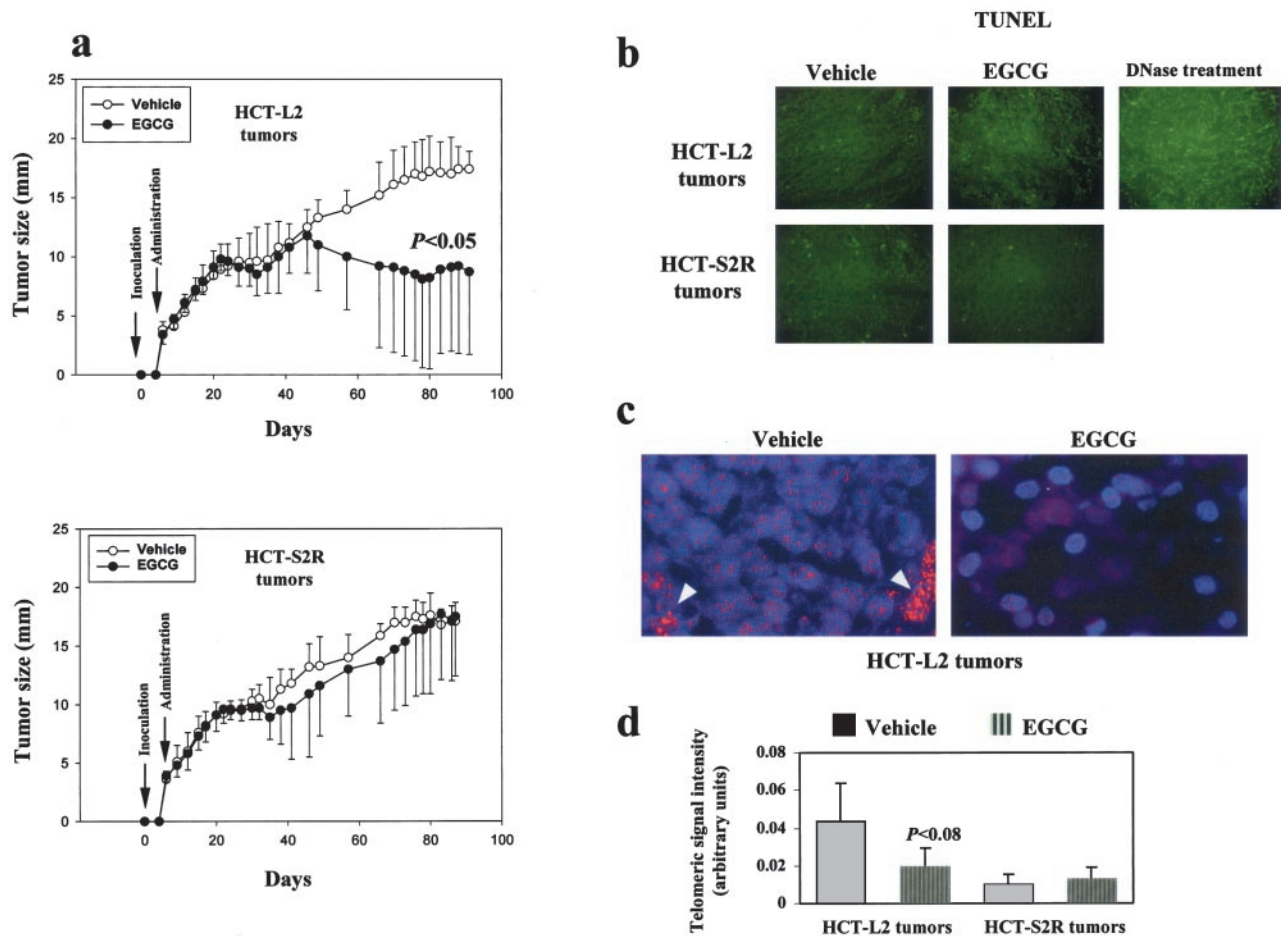
Fig. 5. *a*, effect of prolonged oral administration of EGCG (~1.2 mg/body/day) on the growth of HCT-L2 and HCT-S2R xenografts in BALB/c nude mice. *b*, widespread apoptosis in tissue sections from HCT-L2 group treated with EGCG. *c*, decrease of telomeric signals in tumor sections from EGCG-treated mice. *d*, quantitation of the telomeric signal per nuclear area. Values are the mean of 15 fields (~50 nuclei/field) from four different mice in each treatment group; *bars*, ±SD. *Arrows* indicate the high intensity signals from infiltrating mouse cells.

However, significant body weight loss, cachexia, and end-stage death were observed in the vehicle-treated HCT-L2 group (average body weight 18.8 ± 1.7 g) and in the EGCG or vehicle-treated HCT-S2R groups (average body weight 17.7 ± 1.8 g and 15.5 ± 1.4 g, respectively). TUNEL assay on tumor sections showed a high rate of apoptosis in the EGCG-treated HCT-L2 tumors (Fig. 5*b*). Attempts to detect telomere shortening in excised tumors by standard Southern blot assay were precluded by the presence of murine DNA from infiltrating mouse cells (data not shown). Nevertheless, FISH analysis of telomeres using PNA probes enabled the detection of telomere shortening in tumor areas without murine cell infiltration (Fig. 5, *c* and *d*).

DISCUSSION

The present study uncovered a new property that degraded EGCG and other related polyphenols rather than the intact molecules are very effective in inhibiting the cancer-associated enzyme telomerase. We have reported previously that the inhibition of telomerase by EGCG might provide a plausible explanation for the anticancer effects of dietary polyphenols (13). However, that study was limited only to results demonstrated *in vitro* and without a proper negative control. Here, by using both *in vitro* and *in vivo* models established with telomerase-dependent and -independent cancer cells the present study confirmed the telomerase inhibition-mediated effect of EGCG on cancer. The development of the HCT-S2R cells as a telomerase-

independent but telomerase-positive tumor model may provide a useful tool for studying telomerase inhibitors. It is unlikely that the resistance to telomerase inhibition in HCT-S2R cells is attributable to over-recruitment of telomerase or to residual telomerase activity, because hTR antisense treatment completely inhibited the activity of telomerase in these cells. On the other hand, it is also unlikely that a typical alternative lengthening of telomeres mechanism is involved in the maintenance of telomeres in HCT-S2R, because these cells did not show a heterogeneous telomere length that is characteristic of typical alternative lengthening of telomeres (35). It seems that HCT-S2R cells have evolved certain yet undiscovered auxiliary mechanisms to prevent telomere erosion in the absence of telomerase activity.

Recent studies indicated that telomerase activity inhibition may lead directly to telomere shortening and cellular damage, or it may indirectly, by blocking telomerase-mediated cell survival, enhance cell damage elicited by other factors (20–23). The lag time needed before any noticeable *in vivo* response to EGCG administration is well in agreement with the pharmacodynamics of telomerase inhibition and the accumulative nature of telomere shortening incurred by progressive cell division or by telomere-damaging events suchlike apoptotic signals (36, 37). If the EGCG anticancer effects were mediated by a mechanism other than telomerase inhibition, the onset of tumor response would originate at an earlier course and on both HCT-L2 and HCT-S2R tumors, which was not the case. It is also unlikely that angiogenesis inhibition is a major mechanism for the anticancer

effects of EGCG (38), because tumor neovascularization starts at an early stage before the tumor exceeds 2–3 mm in size (39). In addition, angiogenesis inhibition implies that green tea is severely toxic to various important physiological processes like wound healing or pregnancy (40, 41), whereas it is known that green tea is second only to water as the most popular and safe drink in the world (42). The instability and the prodrug-like behavior of EGCG (and likely many other dietary polyphenols) in body fluids provides a plausible explanation for the yet-unresolved disparity between the concentrations needed to achieve the various acute effects observed *in vitro* (>20 μM) and the plasma levels (<1 μM) at which significant anticancer and chemopreventive effects were observed in animal and epidemiological studies (7, 27). It is worth mentioning that EGCG and other related polyphenols were considered recently as interesting lead compounds that warranted efforts to develop stable derivatives with potent telomerase inhibitory effects (43).

In conclusion, the unique *in vitro* and *in vivo* approaches applied in this study using telomerase-dependent and -independent tumors demonstrated a clear explanation for the anticancer effect of EGCG and other related polyphenols.

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