Effects of green tea extracts on gene expression in HepG2 and Cal-27 cells

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Abstract:

Green tea extract is known to contain compounds that are able to produce antioxidant effects in many types of living cells. Treatment of cultured human hepatoma (HepG2) cells with green tea extract resulted in dramatically increased expression of at least 15 genes that are present on a commercial human drug metabolism gene array. RT-PCR was used to confirm the microarray results, and analysis of the 5'-flanking region of each of these genes revealed potential electrophile/antioxidant response elements. Members of the acetyl transferase, epoxide hydrolase, sulfotransferase and glutathione transferase gene families were strongly induced. In addition, the human tongue carcinoma cell line Cal-27 did not respond to green tea extract in the same way, as none of the induced genes in the HepG2 cells were induced in the Cal-27 cells. The lack of induction of detoxification enzymes in the Cal-27 cell line may help to explain the previously observed increased cytotoxicity of green tea catechins on this cell line.

Keywords: Microarray; Green tea extract; Antioxidant response element; Nrf2; RT-PCR; Gene expression

Article:

1. Introduction

Green tea is one of the most widely used herbal products world-wide and has been linked to a variety of health benefits including anti-inflammatory effects and decreased risk of cardiovascular disease and cancer (Graham, 1992). Considerable effort has been directed toward understanding the molecular basis for these health benefits, and a growing consensus seems to be that green tea contains a variety of polyphenolic compounds that possess anti-oxidant proper-ties. These antioxidant effects may include scavenging reactive oxygen and nitrogen species, inhibition of cellular systems that may produce reactive species, and induction of phase II and anti-oxidant enzymes. Green tea polyphenols (GTP) are believed to initiate the cellular antioxidant response, which results in the induction of a variety of metabolic genes, including human NAD(P)H: quinone oxi-doreductase-1 (NQO1) (Kong et al., 2001), glutathione–S-transferase (GST) (Li and Jaiswal, 1992), heme oxygenase (HO) (Rushmore and Pickett, 1990) and quinone reductase (QR) (Prestera et al., 1993; Alam et al., 1999). The genes for each of these enzymes are known to contain functional electrophile/antioxidant response elements (EpRE/ARE). Chen et al. (2000) also demonstrated that the major green tea catechins, epigalocatechin-3-galate (EGCG) and epicat-echin-3-galate (ECG) were effective at inducing ARE-mediated luciferase activity in HepG2 cells, supporting the notion that the effects of GTE in vivo are the result of EpRE/ARE activation.

The mechanism by which anti-oxidant and phase II drug metabolizing enzymes are induced is very complex, but appears to involve activation of the transcription factor Nrf2 and its subsequent binding to and activation of EpRE/ARE sequences located in the promoter regions of the induced genes (Venugopal and Jaiswal, 1996). Cellular Nrf2 normally resides in the cytosol in complex with a repressor protein Keap1, which sequesters the transcription factor and prevents translocation to the nucleus. Oxidative stress, possibly including the cytosolic accumulation of tea polyphenols and/or hydrogen peroxide, induces dissociation of the Nrf2/Keap1 complex and results in translocation of Nrf2 into the nucleus, where it can heterodimerize with small Maf and bind to its target EpRE/ARE (Itoh et al., 1997, 1999). Other studies suggest that mitogen-activated protein kinases (MAPK) play a role in phosphorylating Nrf2, thus further activating it for gene expression (Yu et al., 1997).

Questions still remain concerning the nature of the EpRE/ARE sequence, thus the full battery of genes controlled via these elements has not been elucidated. Early studies on the EPRE/ARE present in the rat glutathione *S*-transferase Ya subunit gene determined that the consensus sequence 5′-GTGACNNNGC-3′ was required for activity (Rushmore et al., 1991). As more electrophile/anti-oxidant responsive genes were identified, the consensus sequence was refined to include a core sequence 5′– TGANNNNGC-3′ but it was determined that flanking sequences can exert a significant level of control on EpRE/ARE-mediated induction (Wasserman and Fahl, 1997). For example, although the GST-Mu promoter sequence contains the perfect EpRE/ARE consensus sequence 5′-GTGACATAGC-3′, this sequence was ineffective at inducing luciferase reporter gene activity in HepG2 cells, while introducing high AT content into the 5′- and 3′- flanking regions resulted in a functional EpRE/ARE. Although in this instance, A/T content in the 3′-flanking region proved to be an important determinant of EpRE/ ARE-dependent activity, several other putative EpRE/ ARE sequences have been reported that have less than 50% A/T composition in the 3′-flanking region of the core sequence (Katsuoka et al., 2005). Thus identifying antioxidant responsive elements purely on the basis of sequence comparisons is problematic. Several groups have used DNA-Array technology in an attempt to identify potential antioxidant-responsive genes in different cell lines with various antioxidant species. For example, Lee et al. (2003) identified 34 detoxification/antioxidant genes that were induced by tBHQ (a known activator of EpRE/ARE) in Nrf2+ primary cortical astrocytes. Thimmulappa et al. (2002) demonstrated the Nrf2-dependent induction of a host of detoxification/antioxidant genes in mouse intestinal tissue by sulforaphane, a potential chemoprotective agent. Studies of this type have been very informative in terms of identifying targets of Nrf2-mediated induction, but many stop short of identifying potential binding sites for Nrf2 in the promoter regions of the affected genes. The goal of this study was to examine the effects of green tea extract on the expression of various phase I and phase II detoxifying/antioxidant genes in two different human cell lines and to identify potential response elements in the 5′-flanking region of each of the induced genes. The human hepatoma cell line, HepG2, and the oral squamous cell carcinoma line, Cal-27, were used in this study, and the 5′-upstream regions of affected genes were analyzed for putative anti-oxidant response elements.

2. Materials and methods

2. 1. Cell lines and reagents

The human tongue squamous cell carcinoma cell line, CAL-27, was purchased from ATCC (American Type Culture Collection) (Rockville, MD). HepG2 human liver cells were a generous gift from Dr. George Loo (The University of North Carolina at Greensboro, NC). Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, 1% L-glutamine, 50 U/mL penicillin G and 50 μg/mL streptomycin sulfate was used to maintain the CAL 27 cells as monolayer cultures. HepG2 cells were grown under the same conditions as the CAL-27 cells. Green tea extract was produced by Spring Valley (Bohemia, NY) and purchased from a local store. According to the manufacturer, the extract was standardized to contain >50% (by mass) polyphenols, and the content of the four major catechins was determined previously for this extract in this lab to be 24.0% EGCG, 3.4% EC, 19.2% EGC and 7.9% ECG using an HPLC method (Yang and Raner, 2005). Stock solutions of the green tea, extracts were prepared in DMSO.

2.2. Cell culture

HepG2 and CAL 27 cells were grown in 75 cm^2 tissue culture flasks at 37 °C. When the cells were about 80% confluent, they were treated with trypsin and harvested by centrifugation. Cells were counted, and an equal number (1.5 x 10^6 cells/10 mL media) were transferred to each experimental (25 cm^2) flask for treatment with GTE on the following day. Cells were treated with the extracts for 6 h and were subsequently collected and subjected to total RNA isolation, as described in the following section. The vehicle (DMSO) treated cells served as the control group for each experiment. Two independent trials were performed for each experiment, with results that were qualitatively identical.

2.3. Total RNA isolation and RT-PCR analysis

Total RNA from each experimental group of cells was isolated using the SV Total RNA Isolation System protocol (Promega Co., Madison, WI) according to manufacturer's specifications. The protocol involved disruption of cells, denaturation of nucleoprotein complexes, inactivation of endogenous ribonuclease (RNase)

activity and removal of proteins and DNA. Using the First-Strand cDNA Synthesis Kit (Amersham Biosciences Co., Piscataway, NJ) reverse transcription of 10 μg of RNA to a corresponding amount of cDNA was carried out.

2.4. RT-PCR and microarray reactions

cDNA from 10 μg total RNA was used as a template for PCR reactions that were performed in a final volume of 50 μl. The cycling conditions were as follows: mixtures were heated to 95 °C for 4 min and then cycled 30 times through a 1 min denaturation step at 94 °C, a 1 min annealing step at 60 °C, and a 2 min extension step at 72 °C in a Perkin Elmer 9600 DNA cycler (Wellesley, MA). A 4.0 min extension time at 75 °C was included at the end of 30 cycles, and this was followed by incubation at 4 'C until the samples were analyzed. β-actin primers were also used as internal controls. Isolated RNA from vehicle-treated human HepG2 cells was used as a control in each RT-PCR experiment for the phase II enzyme induction. All PCR products were separated by gel electrophoresis using a 1.5% agarose gel and were of the correct size, ensuring that the results reflected amplification of cDNA and not genomic DNA. Primer sequences, expected PCR fragment length, and annealing temperatures for the phase II enzymes examined are listed in Table 1.

Table 1
Primer sequences, expected PCR product sizes, and annealing temperatures for RT-PCR primers used for human CYP isoforms in this study

	Primer sequence	Product
β-actin	5′ GGTCACAACTGCCATCTCG 3′ 5′ GTTCTGCCACTGGTTCACG 3′	202 bp
HAT1	5′ CAGTTCTCAGTCCAACAGGAGGAG 3′ 5′ CGGTCGCAAAGAGCGTAGCTCCA 3′	215 bp
HNMT	5′ GGACAAGAAGCTGCCAGGC 3′ 5′ CTCGAGGTTCGATGTCTTGGC 3′	219 bp
CHAT	5′ GTCTACGCCTGTGGAGCCGATAC 3′ 5′ GGAACCAAGCTTAGTGGCTGGCAGC 3′	255 bp
EPHX1	5′ GGCTTCTCAGAGGCATCCTCC 3′ 5′ CCACATCCCTCTCAGTGAGGCC 3′	273 bp

Using RNA isolated as described under PCR procedures, microarray experiments were carried out using protocols provided with the GEArray Q series human drug metabolism gene array: HS-011.

2.5. Examination of 5'-upstream region of each of the induced genes

The literature supplied with the microarray kits provided genbank references for all of the genes present on the DNA array. Genes that were analyzed in this study include: ATP-Binding Cassette, subfamily C, member 2 (ABCC2, accession no. NM-000392), acetyl-coenzyme A acetyltransferase 1 (ACAT1, Accession no. NM_000019), choline acetyltransferase (CHAT, Accession no. NM _ 020985) microsomal epoxide hydrolase (EPHX1, Accession no. NM_000120), epoxide hydrolase 2 (EPHX2, Accession no. NM _ 001979), histone acetyltranferase 1 (HAT1, accession no. NM003642), MYST histone acetyltransferase 2 (MYST2, accession no. NM_007067), histamine N-methyltransferase (HNMT, Accession no. NM _ 006895), microsomal glutathione-*S*-transferase 1 (MGST1, Accession no. NM _ 020300), microsomal glutathione-*S*-transferase 2 (MGST2, Accession no. NM _ 002413), microsomal glutathione-*S*-transferase 3 (MGST3, Accession no. NM_004528), and sulfotransferase 2A1 (SULT2A1, Accession no. NM_003167). The promoter region for hNQO1 (Accession no. (NM _ 000903) was also obtained and analyzed in the same manner for comparison. Using this information, a BLAST analysis of the human genome was performed for each of the induced genes, and the preceding 5000 base pairs for each gene was obtained. Using a DNA analysis program (BioEdit) obtained from the internet, each of the sequences was analyzed with respect to the presence of the core EpRE/ARE sequence (TGAXXXXGC) as defined by Jaiswal, 2004).

2.6. RT-PCR analysis of Nrf2 mRNA in Cal-27 and HepG2 cells

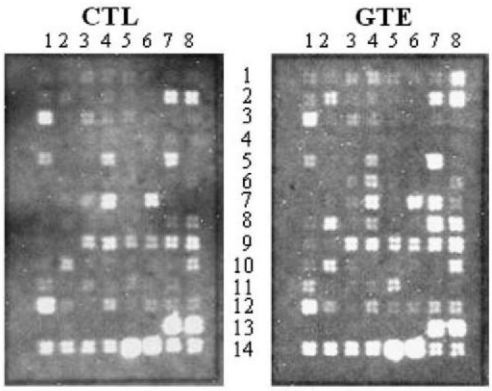
The primers, 5′-ACCCTTGTCACCATCTCAGG-3′ and 5′-TTGCC-ATCTCTTGTTTGCTG-3′ were used to amplify cDNA produced from both HepG2 and Cal-27 cells grown in the presence of 100 μg/mL GTE. These primers were used previously in SCC25 cells to monitor Nrf2 expression (Lin et al., 2002). In the current study

with HepG2 and Cal-27 cells, the Access RT-PCR kit from Promega was used with PCR conditions of 94 °C for 4 min, followed by 30 cycles of 94 °C for 30 s, 57 °C for 30 s, and 72 °C for 1 min. A final extension time of 5 min at 72 °C was used.

3. Results

3.1. Microarray analysis of HepG2 cells treated with GTE

Treatment of HepG2 cells with 100 μg/mL green tea extract resulted in the induction of at least 15 different genes on a human drug metabolism enzyme gene array. Fig. 1 shows the results of the array experiment and includes a table of the genes that were induced significantly by the GTE, along with the location of these genes on the array. The β-actin gene was included on the array in row 14, columns 7 and 8. No increase in β-actin gene expression was observed in the GTE treated cells.



Gene	Row	Column	Fold induction
MRP2	1	4	3.0
ACAT1	1	8	6.3
CHAT	2	2	2.9
EPHX1	6	4	10
GSTA4	6	8	2.5
HAT1	7	7	22
MYST2	7	8	5.2
HNMT	8	2	16.1
NAT5	8	4	3.5
MGST1	8	7	6.2
MGST2	8	8	3.7
SULT1A2	11	1	3.2
SULT2A1	11	5	4.0
UGT2B10	12	7	2.1
UGT2B4	12	8	2.3

Fig. 1. DNA microarray showing induction of phase II genes by GTE. GTE (100 μg/mL) was added to cultured HepG2 cells, which were allowed to grow an additional 6 h following addition of the extract.

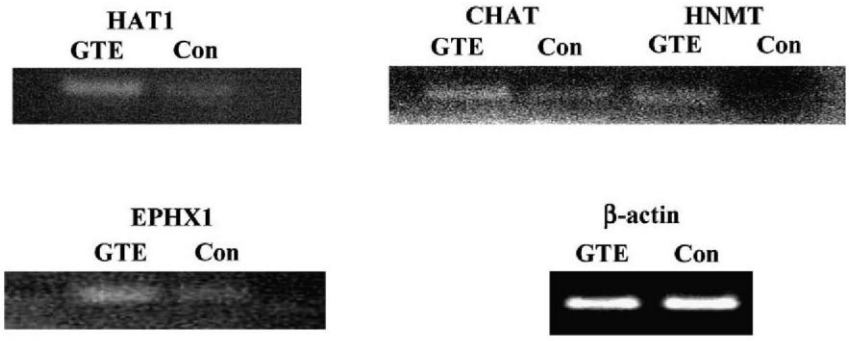


Fig. 2. RT-PCR data confirming induction of five genes. Primer sequences used are shown in Table 1.

3.2. RT-PCR analysis of selected genes in HepG2 treated with GTE

RT-PCR was used to confirm induction of several of the genes for which the array experiment indicated up-regulation. Primers for the HAT1, HNMT, CHAT, and EPHX1 were designed and using mRNA isolated from HepG2 cells treated with GTE or DMSO alone, RT-PCR experiments were carried out (Fig. 2). In each case, visual inspection of the agarose gel, following ethidium bromide staining revealed a significant increase in the intensity of the PCR bands following treatment with GTE. As with the array experiment, β-actin was used as a control, and showed no increase in staining intensity.

3.3. Microarray analysis of Cal-27 cells treated with GTE

The human tongue carcinoma cell line, Cal-27, was also examined by microarray analysis regarding the inducibility of phase II drug-metabolizing enzymes by GTE. Under identical conditions to the HepG2 experiments, the Cal-27 cells were grown and treated with GTE, and RNA was isolated. The microarray results are presented in Fig. 3. Only minor changes in the expression of any of the genes present on the array could be observed, and all of the changes appeared to be decreased expression, in contrast to the results observed for HepG2 cells.

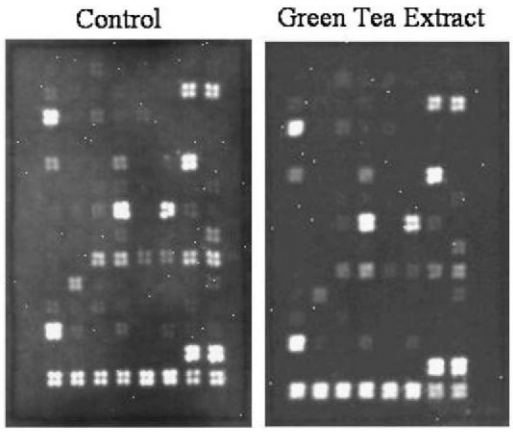


Fig. 3. DNA microarray showing the effect of GTE on cultured Cal-27 cells. GTE (100 μg/mL) was added to cultured cells, which were allowed to grow an additional 6 h following addition of the extract.

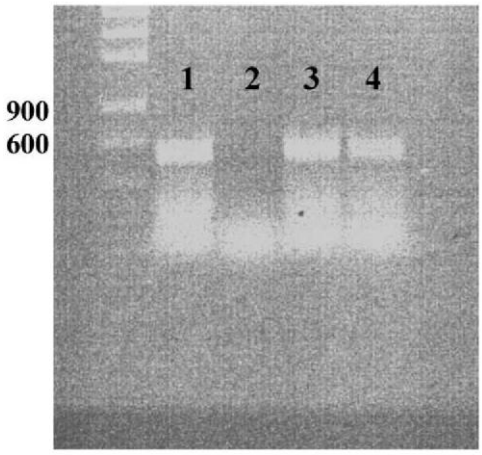


Fig. 4. RT-PCR data using Nrf2 primers for monitoring expression in HepG2 and Cal-27 cells. Lane 1: HepG2 cells. Lane 2: HepG2 cells control with no added reverse transcriptase. Lane 3: Cal-27 cells. Lane 4: Cal -27 cells using 25 cycles instead of 30. The HepG2 and Cal-27 cells were both treated with 100 μg/mL GTE for 6 h.

3.4. RT-PCR analysis of HepG2 and Cal-27 cells using Nrf2 primers

The primers, 5′-ACCCTTGTCACCATCTCAGG-3′ and 5′-TTGCCATCTCTTGTTTGCTG-3′ were used to amplify cDNA produced from both HepG2 and Cal-27 cells grown in the presence of 100 μg/mL GTE. As shown in Fig. 4, the expected RT-PCR band at 544 base pairs was observed in lanes 1 and 3, but not in lane 2, where the reverse transcriptase enzyme was omitted from the HepG2 sample. Densitometer scanning of the resulting bands gave intensities that were within 10% of each other for the Cal-27 and HepG2 cells under identical conditions.

Table 2
Comparison of upstream sequences of several phase II drug metabolizing genes that are induced by antioxidants with the functional ARE sequence in hNQO

hNQO		
	−530	— cc**gcagt**caca**gtgac**tca**gc**agaatctg—
EPHX1		
	−1377	— gtgccctct**tgac**tga**gc**ctcgagc—
	−14	— caca**catgag**attggc**tgac**ttg**gc**a—
	−2593	— ccctt**gatgctgag**aat**gc**accttctcc—
	−1667	— gtg**gatgtagggg**t**gatgc**a**gc**cctgctc—
mGST1		
	−633	— ggacatc**gtgac**aaa**gc**aaattgt—
	−4509	— tccgtcat**gtgag**gaa**gc**agcaggaa—
	−1544	— agcttgca**gtgagctgag**atc**gc**gcca—
	−3359	— gcct**tgaggtctgtgat**tca**gc**ttgaattttt—
mGST2		
	−4520	— accctggaagt**tgaggctgc**agtgag—
	−418	— ctgca**gtgagctatgat**cgt**gc**caccacacac—

Table 3
Six different sequences in the upstream region of mGST3 gene that match the consensus ARE core sequence TGAXXXXGC

mGST3		
	−3079	— gaaaacaatca**tgac**ata**gc**aatgtctgagaat—
	−255	— ccgggaggta**tgac**ctg**gc**tgggacagttta—
	−3018	— atggtgacctt**tgagagagc**agtgtgagatg—
	−1433	— aaatttttttt**gagac**t**gc**agtctccggt—
	−1409	— ccggtgtaaaa**tgaggg**t**gc**aaggtgcatc—
	−117	— ccaaccagtggccc**tgagttggc**aacttag—
	−4400	— aatatagtag**gtgatt**ct**gc**atgaaacagaaac—

4. Discussion

The effect of GTE on cultured human hepatoma cells (HepG2) was to cause an increase in the mRNA of a wide variety of phase II drug metabolizing enzymes. Several of the genes that were induced by GTE in the current study are known to respond to other electrophiles and antioxidants via activation of antioxidant response

elements in the adjacent 5′-region of the genes. For example, the microsomal glutathione *S*-transferase 1 promoter can be activated by menadione and paraquot as shown by Kelner et al. (2000) using a luciferase reporter gene construct in HepG2 cells. The mGST1 promoter region contains an exact copy of the consensus EpRE/ARE sequence at position —490 as shown in Table 2. It has been demonstrated that green tea extract contains compounds that are able to elicit an antioxidant response in cultured human cell lines and in live animals (Chou et al., 2000).

The array data from this study indicates that the MGST1 gene is induced dramatically by exposure of HepG2 cells to green tea extract. The most likely mechanism for this induction involves Nrf2-mediated activation of the EpRE/ARE present in the MGST1 promoter region. In addition, MGST2 but not MGST3 was dramatically induced by GTE. Analysis of the 5′-upstream region of these two genes revealed several potential EpRE/ARE sequences. In MGST2, a sequence at —413, TGATCGTGC, matched the consensus core sequence defined by Jaiswal (2004). An additional remote sequence at position 4508, TGAGGCTGC, could also be considered a candidate ARE site. Induction of microsomal epoxide hydrolase 1 (EPHX1) by sulforaphane in an Nfr2-dependent manner was also observed in the same study involving Nrf2 knockout mice, consistent with the presence of an ARE sequence. The array data using GTE as an inducer in HepG2 cells in the current study supported this idea, and several potential EpRE/ARE sites in the upstream region of the human EPHX1 gene were identified, as shown in Table 2. Analysis of the related cytosolic EPHX2 promoter yielded no similar sequences, consistent with the lack of induction observed (see Table 3).

Table 4
Potential ARE sequences and positions in several genes that were induced by GTE in this study

HAT 1		
	−3375	—— c**cagt**gtg**gtgac**tggg**c**agtactattat—-
	−2388	—— gtctcgctctct**tgag**cagg**c**tggagtgcagt—
	−1189	—— atctctaaaa**tgag**aggg**c**gtgttagtt—-
HNMT		
	−4233	—- aaaaattgttg**tgag**agt**gc**tagtcctg—
	−198	—- gctgtagatttc**tgag**agt**gc**tgtgtag—
ACAT1		
	−4179	—- tccagcctgg**gtgac**agag**c**aaggctct—
	−1053	—- tgagaccaggagtt**tgag**gct**gc**agtgagatat—
	−2799	—- tggtgctcatattg**gtgat**aaag**c**tactcttatttttt-
CHAT		
	−3125	— gaagagcgggcgc**tgac**ccc**gc**gagtcctagaac-
	−4934	— aagggctgca**gtgag**tggg**c**tctcacctc-
	−1960	— gctagtgaacccct**tgag**cggg**c**ccttcatcgac-
	−1670	— gcatcctcta**tgag**ttc**gc**cggcaag——
Sult2A1		
	−1590	— gacttattagcaaca**tgac**ctg**gc**actaatgatg—

A number of genes were also induced by GTE that have not previously been associated with an EpRE/ARE. Those genes along with potential EpRE/ARE sequences are listed in Table 4. Of particular note are the hi stone acetyltransfer-ase1 (HAT1), acetyl-coenzyme A acetyltransferase 1 (ACAT1), choline acetyltransferase (CHAT), and histamine N-methyltransferase (HNMT) genes. Each of these genes appeared to be dramatically activated by GTE in the HepG2 cells. Likewise, several copies of the core EpRE/ARE sequence were identified in the 5′-upstream regions of the genes. There was a striking similarity between the putative HAT1 EpRE/ARE at —3374 and the functionally established EpRE/ARE for hNQO at position — 530. Two additional sequences closer to the gene start site were also identified as potential response element sites as well. Very little information is available regarding HAT1 expression and inducibility. Although this putative EpRE/ARE is considerably farther from the transcription start site than in hNQO, it is within a reasonable distance to effect transcription. Although a role for HAT1 in processing newly synthesized histones has been established, in yeast it is apparently not essential, as knockout experiments yielded no phenotypical differences from wild-type cells (Kleff et al., 1995).

In addition to HAT1, the HNMT gene, as well as the genes for CHAT and ACAT1, were strongly induced by GTE treatment. As with HAT1, each gene possessed at least one potential EpRE/ARE sequence in the 5'-region. It should be noted that a number of genes included in this array contained core ARE sequences but were not induced. For example, ACAT2, which is closely related to ACAT1 contains six sites with the correct motif, including one at —876, GTGACAAAGCTTTCTTGTT that closely matches the hNQO ARE, yet induction of this gene was marginal. MGST3 contained seven sequences that match the core ARE sequence, but this isozyme was not induced.

No induction was observed in Cal-27 human tongue carcinoma cells. A possible explanation for this observation would be a lack of expression of Nrf2 in Cal-27 cells, since Nrf2 has been implicated in the antioxidant response. To test this hypothesis, RT-PCR experiments were performed in which Cal-27 and HepG2 cells treated with 100 mg/mL GTE were examined for their ability to produce Nrf2 mRNA. Both cell lines appeared equally capable of producing this transcription factor. Alternatively, factors expressed in Cal-27 cells but not HepG2 cells could silence the AREs. This is consistent with the finding of Weisburg et al. (2004) that exposure of Cal-27 cells to EGCG results in a decrease in the amount of cellular GSH, presumably through down-regulation of glutamate cysteine ligase (GCL), the rate limiting enzyme in GSH synthesis. The differential expression patterns in HepG2 cells and Cal-27 cells in response to GTE and GTE catechins may help to explain differences in cytotoxicity of GTE toward the two cell lines as well. Cal-27 cells were shown to be very sensitive to EGCG, relative to other human cell lines, and it is likely that their inability to respond by producing phase II detoxifying enzymes is at least partially responsible (Weisburg et al., 2004).

Although the induced genes reported in this research showed very clear induction, we cannot rule out the possibility that other genes that are poorly expressed in this cell line were induced, but were still below the limit of detection. For example, recent RT-PCR studies on the effect of GTE on drug metabolizing enzymes in HepG2 cells and Cal-27 cells indicated that several Cytochrome P450 isoforms showed modest induction by GTE, including P450 2E1, 1A1, 1A2, and 2C9 in both cell lines (Yang and Raner, 2005). Furthermore, the individual catechins did not induce the P450 enzymes, only the complete extract. In light of the current data, it is reasonable to conclude that the mechanism involved in the induction of phase II enzymes in HepG2 cells by GTE is different from that of the cytochrome P450 induction. Of the isoforms identified by RT-PCR, only P4501A1 could be observed in the array experiment, and apparent expression levels were very low, even for this isoform. It is known that cytochrome P450 expression in HepG2 is very low, and it was noted in the RT-PCR experiments that additional PCR cycles were needed to observe gene expression. We there-fore cannot rule out induction of poorly expressing genes on the basis of this array experiment. In fact, a number of genes, including P4501A1 appear to increase in the GTE treated cells, however the effects are not as dramatic as seen with the 15 genes noted herein.

There is still debate regarding the actual molecular species that is responsible for the anti-oxidant properties of GTE, and in some instances questions have been raised as to whether GTE has anti-oxidant properties (Elbling et al., 2005). As demonstrated in this work and the work of others (Nakagawa et al., 2002, 2004), the choice of cell lines and conditions used appear to have a dramatic impact on the results obtained, and conclusions reached. The amount of hydrogen peroxide generated is dependent on a number of variables including the presence of certain metal ions, the concentration and identity of catechins present, the pH of the solution, the presence of cells in the solution, and the buffering agents used (Hayakawa et al., 2004). At high catechin concentration (>20 μM) the H_2O_2 generated can be quantified and appears to have oxidant/cytotoxic effects on the cell. Elbling et al. (2005) and others have suggested that at nanomolar concentrations of catechins, it is likely that the amount of H_2O_2 produced is very low, but enough to trigger an anti-oxidant response in the cell, giving rise to the antioxidant properties of GTE. Furthermore, Vital et al. (2004) have demonstrated using DNA microarray analysis, that even in the presence of catalase, EGCG still produces an inductive effect on certain signaling pathways in human bronchial epithelial cells, indicating the catechins themselves have some inherent ability to induce gene transcription apart from their H_2O_2 generating ability.

Although it is not possible to say whether the catechins themselves, or H_2O_2 generated in the cell culture system, produced the effects observed in the array data, the results are consistent with a general antioxidant response to GTE in HepG2 cells, but not in Cal-27 cells. Furthermore, several new GTE-responsive genes have been identified and potential ARE sequences have been located in the 5′- upstream regions of all of these genes. As more functional ARE sequences are identified, it will be possible to gain a better understanding of the true nature of this element and the additional factors responsible for regulating anti-oxidant gene expression.

References

Alam, J., Stewart, D., Touchard, C., Boinapally, S., Choi, A.M., Cook, J.L., 1999. Nrf2, a cap'n'collar transcription factor, regulates induction of the heme oxygenase-1 gene. J. Biol. Chem. 274, 26071–26078.

Chen, J., Yu, R., Owuor, E.D., Kong, A.N., 2000. Activation of antioxidant-response element (ARE), mitogen-activated protein kinases (MAPKs) and caspases by major green tea polyphenol components during cell survival and death. Arch. Pharm. Res. 23, 605–612.

Chou, F.-P., Chu, Y.-C., Hsu, J.-D., Chiang, H.-C., Wang, C.-J., 2000. Specific induction of glutathione s-transferase subunit expression by epigallocatechin gallate in rat liver. Biochem. Pharmacol. 60, 643–650.

Elbling, L., Weiss, R.-M., Teufelhofer, O., Uhl, M., Knasmueller, S., Schulte-Hermann, R., Berger, W., Micksche, M. Green tea extract and (-)-epigallocatechin-3-gallate, the major tea catechin, exert oxidant but lack antioxidant activities, Faseb J. Express 10.1096/fj.04-2915fje, published online February 28, 2005.

Graham, H.N., 1992. Green tea composition, consumption, and polyphenol chemistry. Prev. Med. 21, 334–350.

Hayakawa, F., Ishizu, Y., Hoshino, N., Yamaji, A., Ando, T., Kimura, T., 2004. Prooxidative activities of tea catechins in the presence of Cu2+. Biosci. Biotechnol. Biochem. 68, 1825–1830.

Itoh, K., Chiba, T., Takahashi, S., Ishii, T., Igarashi, K., Katoh, Y., Oyake, T., Hayashi, N., Satoh, K., Hatayama, I., Yamamoto, M., Nabeshima, Y., 1997. An Nrf2/small Maf heterodimer mediates the induction of phase II detoxifying enzyme genes through antioxidant response elements. Biochem. Biophys. Res. Commun. 236, 313–322.

Itoh, K., Wakabayashi, N., Katoh, Y., Ishii, T., Igarashi, K., Engel, J.D., Yamamoto, M., 1999. Keap1 represses nuclear activation of antioxidant responsive elements by Nrf2 through binding to the amino-terminal Neh2 domain. Genes Dev. 13, 76–86.

Jaiswal, A.K., 2004. Nrf2 signaling in coordinated activation of antioxidant gene expression. Free Rad. Biol. Med. 36, 1199–1207.

Katsuoka, F., Motohashi, H., Engal, J.D., Yamamoto, M., 2005. Nrf2 transcriptionally activates the mafG gene through an antioxidant response element. J. Biol. Chem 280, 4483–4490.

Kelner, M.J., Bagnell, R.D., Montoya, M.A., Estes, L.A., Forsberg, L., Morgenstern, R., 2000. Structural organization of the microsomal glutathione *S*-transferase gene (MGST1) on chromosome 12p13.1– 13.2. Identification of the correct promoter region and demonstration of transcriptional regulation in response to oxidative stress. J. Biol. Chem. 275, 13000–13006.

Kleff, S., Andrulis, E.D., Anderson, C.W., Sternglanz, R., 1995. Identification of a gene encoding a yeast histone H4 acetyltransferase. J. Biol. Chem. 270, 24674–24677.

Kong, A.N., Owuor, E., Yu, R., Hebbar, V., Chen, C., Hu, R., Mandlekar, S., 2001. Induction of xenobiotic enzymes by the MAP kinase pathway and the antioxidant or electrophile response element (ARE/EpRE). Drug Metab. Rev. 33, 255–271.

Lee, J.M., Calkins, M.J., Chan, K., Kan, Y.W., Johnson, J.A., 2003. Identification of the NF-E2-related factor-2-dependent genes confer-ring protection against oxidative stress in primary cortical astrocytes using oligonucleotide microarray analysis. J. Biol. Chem. 278, 12029– 12038.

Li, Y., Jaiswal, A.K., 1992. Regulation of human NAD(P)H: quinone oxidoreductase gene. Role of AP1 binding site contained within human antioxidant response element. J. Biol. Chem. 267, 15097–15104.

Lin, R., Nagai, Y., Sladek, R., Bastien, Y., Ho, J., Petrecca, K., Sotiropoulou, G., Diamandis, E.P., Hudson, T.J., White, J.H., 2002.
Expression profiling in squamous carcinoma cells reveals pleiotropic effects of vitamin D3 analog EB1089 signaling on cell proliferation, differentiation, and immune system regulation. Mol. Endocrinol. 16, 1243–1256.

Nakagawa, H., Wachi, M., Woo, J.-T., Kato, M., Kasai, S., Takahashi, F., Lee, I.-S., Nagai, K., 2002. Fenton reaction is primarily involved in a mechanism of (-)-epigallocatechin-3-gallate to induce osteoclastic cell death. Biochem. Biophys. Res. Commun. 292, 94–101.
Nakagawa, H., Hasumi, K., Woo, J.-T., Nagai, K., Wachi, M., 2004. Generation of hydrogen peroxide primarily contributes to the induction of Fe(II)-dependent apoptosis in Jurkat cells by (-)-epigallocatechin gallate. Carcinogenesis 25, 1567–1574.
Prestera, T., Holtzclaw, W.D., Zhang, Y., Talalay, P., 1993. Chemical and molecular regulation of enzymes that detoxify carcinogens. Proc. Natl. Acad. Sci. USA 90, 2965–2969.
Rushmore, T.H., Pickett, C.B., 1990. Transcriptional regulation of the rat glutathione *S*-transferase Ya subunit gene. Characterization of a xenobiotic-responsive element controlling inducible expression by phenolic antioxidants. J. Biol. Chem. 265, 14648–14653.
Rushmore, T.H., Morton, M.R., Pickett, C.B., 1991. The antioxidant responsive element: activation by oxidative stress and identification of the DNA consensus sequence required for functional activity. J. Biol. Chem. 266, 11632–11639.
Thimmulappa, R.K., Mai, K.H., Srisuma, S., Kensler, T.W., Yamamoto, M., Biswal, S., 2002. Identification of Nrf2-regulated genes induced by the chemopreventive agent sulforaphane by oligonucleotide micro-array. Cancer Res. 62, 5196–5203.
Venugopal, R., Jaiswal, A.K., 1996. Nrf1 and Nrf2 positively and c-fos and Fra1 negatively regulate the human antioxidant response element-mediated expression of NAD(P)H: quinone oxidoreductase1 gene. Proc. Natl. Acad. Sci. USA 93, 14960–14965.
Vital, R., Selvanayagam, Z.E., Sun, Y., Hong, J., Liu, F., Chin, K.-V., Yang, C.S., 2004. Gene expression changes induced by green tea polyphenol (-)-epigallocatechin-3-gallate in human bronchial epithelial 21BES cells analyzed by DNA microarray. Mol. Cancer Ther. 3, 1091–1099.
Wasserman, W., Fahl, W.E., 1997. Functional antioxidant responsive elements. Proc. Natl. Acad. Sci. USA 94, 5361–5366.
Weisburg, J.H., Weissman, D.B., Sedaghat, T., Babich, H., 2004. In vitro cytotoxicity of epigallocatechin gallate and tea extracts to cancerous and normal cells from the human oral cavity. Basic Clin. Pharmacol. Toxicol. 95, 191–200.
Yang, S.-P., Raner, G.M., 2005. Cytochrome P450 expression and activities in human tongue cells and their modulation by green tea extract. Toxicol. Appl. Pharmacol. 202, 140–150.
Yu, R., Jiao, J.J., Duh, J.L., Gudehithlu, K., Tan, T.H., Kong, A.-N.T., 1997. Activation of mitogen-activated protein kinases by green tea polyphenols: potential signaling pathways in the regulation of antioxidant-responsive element-mediated phase II enzyme gene expression. Carcinogenesis 18, 451–456.