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Research Paper

Comparison of (–)-Epigallocatechin-3-Gallate Elicited Liver and Small Intestine Gene Expression Profiles Between C57BL/6J Mice and C57BL/6J/Nrf2 (–/–) Mice

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Purpose. This study was conducted to study global gene expression profiles elicited by (–)-epigallocatechin-3-gallate (EGCG) in mouse liver and small intestine, as well as to identify EGCG-regulated Nrf2-dependent genes.
Methods. C57BL/6J and C57BL/6J/Nrf2(–/–) mice were given an oral dose of EGCG at 200 mg/kg or treated with vehicle. Both liver and small intestine were collected 3 h and 12 h after treatment. Total RNA was extracted from the tissues and gene expression profiles were analyzed using Affymetrix mouse genome 430 2.0 array and GeneSpring 6.1 software. Microarray data were validated using quantitative real-time reverse transcription-PCR chain reaction analysis.
Results. Genes that were either induced or suppressed more than two fold by EGCG treatment compared with vehicle treatment in the same genotype group were filtered using the GeneSpring software. Among these well-defined genes, 671 EGCG - regulated Nrf2-dependent genes and 256 EGCG - regulated Nrf2-independent genes were identified in liver, whereas 228 EGCG - regulated Nrf2-dependent genes and 98 EGCG - regulated Nrf2-independent genes were identified in the small intestine. Based on their biological functions, these genes mainly fall into the category of ubiquitination and proteolysis, electron transport, detoxification, transport, cell growth and apoptosis, cell adhesion, kinase and phosphatases, and transcription factors.
Conclusions. Genes expressed in mouse liver are more responsive to oral treatment of EGCG than those expressed in small intestine. EGCG could regulate many genes in both organs in an Nrf2-dependent manner. The identification of genes related to detoxification, transport, cell growth and apoptosis, cell adhesion, kinase, and transcription regulated by EGCG not only provide potential novel insight into the effect of EGCG on global gene expression and chemopreventive effects, but also point to the potential role of Nrf2 in these processes.

KEY WORDS: chemoprevention; (–)-epigallocatechin-3-gallate; global gene expression profile; microarray; nuclear factor E_2-related factor 2.

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ABBREVIATIONS: ABC, ATP-binding cassette; ALOX, arachidonate 12-lipoxygenase; ARE, antioxidant response element; DNMT, DNA methyltransferases; EGCG, (–)-epigallocatechin-3-gallate; IGF-1R, insulin-like growth factor 1 receptor gene; MAPK, mitogen-activated protein kinase; MMP, matrix metalloprotease; NF-κB, nuclear factor kappa B; Nrf2, nuclear factor E_2-related factor 2; NOS, nitric oxide synthase.

INTRODUCTION

The medicinal benefits of drinking green tea have been known in Asian countries since ancient times. From the *in vivo* animal cancer model studies, (–)-epigallocatechin-3-gallate (EGCG) or green tea extract has been shown to inhibit tumorigenesis on different organ sites (1). These include 7,12-dimethylbenz[*a*]anthracene (DMBA)-initiated skin tumor (2) in sensitive to mouse carcinogenesis (SENCAR); UVB radiation-induced photocarcinogenesis in SKH-1 hairless mice (3,4); lung tumorigenesis (5) in A/J mice; prostate tumorigenesis in athymic mice (6) and transgenic murine prostate cancer model (TRAMP) mice (7); breast tumor xenograft in athymic mice (6,8); and DMBA initiation in the Sprague–Dawley (S–D) rat model (9). During the past two decades, many case-control and cohort epidemiological studies have been conducted to investigate the effects of green tea consumption on the incidence of different types of human cancer, including stomach cancer (10,11) pancreatic cancer (12), colorectal cancer (12), lung cancer (13), breast cancer (14,15), prostate cancer (16), and ovarian cancer (17). The epidemiologic studies on tea drinking and stomach cancer, however, are inconclusive (18). In addition to its possible cancer chemoprevention effects, green tea consumption has also been shown to reduce the risk of cardiovascular disease (19) and to protect against coronary atherosclerosis in men (20).

Inspired by these findings, many studies have been carried out to unravel the protective mechanisms of green tea and especially the major polyphenol constituent, EGCG, by using *in vitro* cell culture and *in vivo* rodent cancer models. Several molecular mechanisms in the anticarcinogenesis effects of EGCG have been implicated (21). EGCG could cause G_1 cell cycle arrest by inducing the expression of cyclin-dependent kinase inhibitors and downregulating hyperphosphorylated pRb protein (22,23), and subsequently induce cancer cell apoptosis through mitochondrial pathway, thereby increasing the ratio of Bax/Bcl-2 (24). EGCG could also inhibit cancer cell invasion and metastasis by downregulating matrix metalloproteinases (MMPs) and increasing the cell adhesion function (25,26). Additionally, the inhibitory effects of EGCG on lipoxygenase (LOX)-dependent arachidonic acid metabolism (27), fatty acid metabolism (28,29), and NOS (30) are not only related to its cancer prevention effect but may also be related to its protective effects against cardiovascular disease. In terms of regulating cellular signaling pathways, EGCG has been shown to inhibit many tumor-associated signaling pathways, including transforming growth factor-beta pathway (TGF-β) (31), vascular endothelial growth factor receptor (VEGFR) pathway (32), epidermal growth factor receptor (EGFR) pathway (33–35), platelet-derived growth factor (PDGF) pathway (36,37), NF-κB (30), AP-1, PI3K/Akt, and MAPK pathways (26,38,39).

Basic leucine zipper family transcription factor nuclear factor E_2-related factor 2 (Nrf2) is involved in the regulation of antioxidant response element (ARE)-mediated gene transcription (40). ARE is an *cis*-acting element [5′-(G/A)TGA(G/C)nnnGC(G/A)-3′] found in the 5′-flanking region of many phase II drug metabolizing/detoxifying enzyme genes such as glutathione *S*-transferase (GST), UDP-glucuronosyltransferase (UGT), and NAD(P)H: quinone oxidoreductase-1 (NQO1) (41). Under a homeostatic condition, Nrf2 is sequestered in the cytoplasm by Kelch-like ECH-associated protein (Keap1) (42). Exposure of cells to oxidative stress or phase II gene/ARE inducers will trigger the release of Nrf2 from Keap1 and facilitates the nuclear translocation of Nrf2 (43). The nuclear translocation of Nrf2 and subsequent dimerization with small Maf-F/G/K protein and coactivators such as cAMP response element binding protein (CREB)-binding protein (CBP) will drive the transcriptional activation of its target genes (44). Phase II detoxification enzyme and antioxidant enzyme genes are the main targets of Nrf2/ARE-mediated gene transcription, and therefore Nrf2 is believed to play an important role in cancer chemoprevention and regarded as a potential molecular target of cancer chemoprevention (41). To support this role, studies in Nrf2-deficient mice have shown that phase II enzyme expression was dramatically attenuated in the Nrf2 knockout mice, and these mice were also much more susceptible to carcinogen-induced carcinogenesis (45,46). Because EGCG induced ARE-mediated gene expression in our previous study (47), the interaction of Nrf2-mediated signaling pathway, together with other mechanisms described above, may contribute to the overall chemopreventive function of EGCG.

In the current study, the global gene expression profiles elicited by oral administration of EGCG in wild-type and Nrf2 knockout mice were compared by microarray analysis. In addition to genes that were regulated by EGCG, regardless of the Nrf2 status, clusters of Nrf2-dependent genes regulated by EGCG were also identified. The identification of these genes will give us some valuable insights in the potential role of Nrf2 in the EGCG-mediated gene regulation. The current study is also the first to investigate the global gene expression profiles elicited by EGCG in the *in vivo* mouse model where the role of Nrf2 is examined.

MATERIALS AND METHODS

Animal and Treatment

Nrf2 knockout mice Nrf2 (–/–) (C57BL/SV129) were described previously (48). Nrf2 (–/–) mice were backcrossed with C57BL/6J mice (Jackson Laboratory, Bar Harbor, ME USA). DNA was extracted from the tail of each mouse and genotype of the mouse was confirmed by polymerase chain reaction (PCR) by using primers (3′-primer, 5′-GGA ATG GAA AAT AGC TCC TGC C-3′; 5′-primer, 5′-GCC TGA GAG CTG TAG GCC C-3′; and lacZ primer, 5′-GGG TTT TCC CAG TCA CGA C-3′). Nrf2 (–/–) mice-derived PCR products show only one band of ~200 bp, Nrf2 (+/+) mice-derived PCR products showed a band of ~300 bp, whereas both bands were shown in Nrf2 (+/–) mice PCR products. Male C57BL/6J/Nrf2(–/–) mice from third generation of backcrossing were used in this study. Age-matched male C57BL/6J mice were purchased from Jackson Laboratory. Mice between 9 and 12 weeks old were used and housed at Rutgers Animal Facility with free access to water and food under 12 h light/dark cycles. After 1 week of acclimatization, mice were put on AIN-76A diet (Research Diets Inc., New Brunswick, NJ, USA) for another week. Mice were then treated with EGCG (LKT Laboratories Inc., St. Paul, MN, USA) at a dose of 200 mg/kg (dissolved in 50% PEG 400 solution at concentration of 20 mg/mL) by oral gavages. The

control groups were given vehicle only (50% PEG 400 solution). Each treatment was administrated to a group of four animals for both C57BL/6J and C57BL/6J/Nrf2(–/–) mice. Mice were sacrificed at 3 and 12 h after EGCG treatments or 3 h after vehicle treatment (control group). Livers and small intestines were removed and stored in RNA Later (Ambion, Austin, TX, USA) solution.

Sample Preparation for Microarray Analyses

Total RNA from liver and small intestine tissues were isolated by using a method of TRIzol (Invitrogen, Carlsbad, CA, USA) extraction coupled with the RNeasy kit from Qiagen (Valencia, CA, USA). Briefly, tissues were homogenized in trizol and then extracted with chloroform by vortexing. A small volume (1.2 mL) of aqueous phase after chloroform extraction and centrifugation was adjusted to 35% ethanol and loaded onto an RNeasy column. The column was washed, and RNA was eluted following the manufacturer's recommendations. RNA qualities were examined by electrophoresis, and concentrations were determined by UV spectrometry.

Microarray Hybridization and Data Analysis

Affymetrix (Affymetrix, Santa Clara, CA, USA) mouse genome 430 2.0 array was used to probe the global gene expression profile in mice following EGCG treatment. The mouse genome 430 2.0 Array is a high-density oligonucleotide array that comprised over 45,101 probe sets representing over 34,000 well-substantiated mouse genes. The library file for the array is available at http://www.affymetrix.com/support/technical/libraryfilesmain.affx. After RNA isolation, all the subsequent technical procedures including quality control and concentration measurement of RNA, cDNA synthesis and biotin-labeling of cRNA, hybridization, and scanning of the arrays were performed at Cancer Institute of New Jersey (CINJ) Core Expression Array Facility of Robert Wood Johnson Medical School (New Brunswick, NJ). Each chip was hybridized with cRNA derived from a pooled total RNA sample from four mice per treatment group, per time point, per organ, and per genotype (a total of 12 chips were used in this study) (Fig. 1). Briefly, double-stranded cDNA was synthesized from 5 μg of total RNA and labeled using the ENZO BioArray RNA transcript labeling kit (Enzo Life Sciences, Inc., Farmingdale, NY, USA) to generate biotinylated cRNA. Biotin-labeled cRNA was purified and fragmented randomly according to Affymetrix's protocol. A total of 200 μL of sample cocktail containing 15 μg of fragmented and biotin-labeled cRNA was loaded onto each chip. Chips were hybridized at 45°C for 16 h and washed with fluidics protocol EukGE-WS2v5 according to Affymetrix's recommendation. At the completion of the fluidics protocol, the chips were placed into the Affymetrix GeneChip Scanner, where the intensity of the fluorescence for each feature was measured. The expression value (average difference) for each gene was determined by calculating the average of differences in intensity (perfect match intensity – mismatch intensity) between its probe pairs. The expression analysis file created from each sample (chip) was imported into GeneSpring 6.1 (Silicon Genetics, Redwood City, CA, USA) for further data characterization. Briefly, a new experiment was generated after importing data from the same organ in which data were normalized by the array to the 50th percentile of all measurements on that array. Data filtration based on flags present in at least one of the samples was first performed, and a corresponding gene list based on those flags was generated. Lists of genes that were either induced or suppressed more that two fold between treated *vs.* vehicle group of same genotype were created by filtration-on-fold function within the presented flag list. By using color-by-Venn-Diagram function, lists of genes that were regulated more than two fold only in C57BL/6J mice in both liver and small intestine were created. Similarly, lists of gene that were regulated over two fold regardless of genotype were also generated.

Quantitative Real–Time PCR for Microarray Data Validation

To verify the microarray data, several genes (including the housekeeping gene GAPDH) from different categories were chosen for quantitative real-time PCR analyses. The specific primers for these genes were designed by using Primerexpress software (Applied Biosystems, Foster City, CA, USA) and listed in Table I. Instead of using pooled RNA from each group, RNA samples isolated from individual mouse as described above were used in real-time PCR analyses. For real-time PCR, the following procedure was followed: briefly, first-strand cDNA was synthesized using 4 μg of total RNA following the protocol of SuperScript III First-Strand cDNA Synthesis System (Invitrogen) in a 40-μl reaction volume. PCR reactions were carried out using 100 times diluted cDNA product, 60 nM of each primer, and SYBR Green master mix (Applied Biosystems) in 10 μl reactions. The PCR parameters were set using SDS 2.1 software (Applied Biosystems) and involved the following stages: 50°C for 2 min, 1 cycle; 95°C for 10 min, 1 cycle; 95°C for 15 s → 55°C for 30 s → 72°C for 30 s, 40 cycles; and 72°C for 10 min, 1 cycle. Levels of quantitative reverse transcription product were measured using SYBR Green fluorescence collected during real-time PCR on an Applied Biosystems PRISM 7900HT system. A control cDNA dilution series was created for each gene to establish a standard curve. After conclusion of the reaction, dissociation curve analysis was performed using the SDS 2.1 software to ascertain the integrity of the PCR reaction product and the absence of primer dimers. Gene expression was determined by normalization with control gene GAPDH. The correlation between the corresponding microarray data and the real-time PCR data was validated via Spearman rank correlation.

RESULTS

EGCG–Altered Gene Expression Pattern in Mouse Liver and Small Intestine

After data normalization, 58.3% (26,289) of the probes passed the filtration based on flags present in at least one of the six liver sample arrays. Among these probes, about 8.6–10.2% of them were either induced or suppressed over two fold by EGCG regardless of genotype and treatment time. Moreover, there was no large difference in the number of probes being regulated by EGCG between C57BL/6J and C57BL/6J/Nrf2(–/–) groups or between different time points. Expression levels of 671 well-defined genes were either

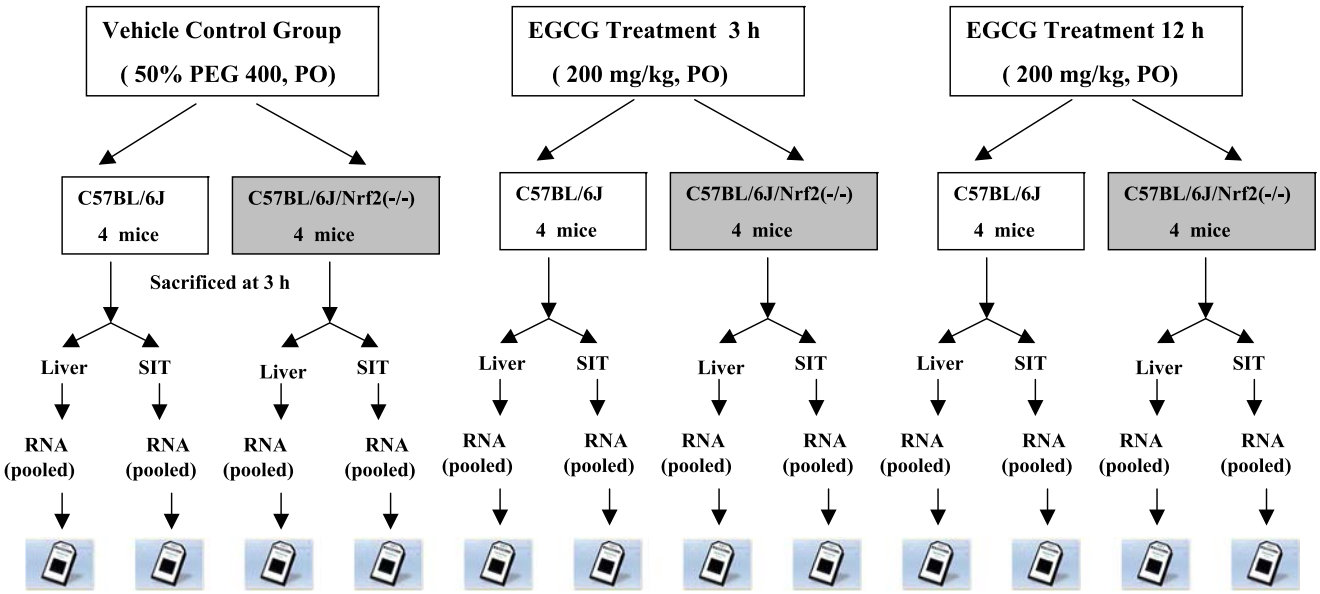

Fig. 1. Schematic representation of experimental design.

induced (554) or suppressed (117) over two fold by EGCG only in the wild-type mice at both time points, whereas 256 well-defined genes were either induced (205) or suppressed (51) over two fold by EGCG in the liver of both genotype groups (Fig. 2A). Similar changes in gene expression profiles were also observed in small intestine. Overall, the expression levels of 61.7% (27,815) probes were detected at least in one of the small intestine sample arrays. Compared with the results from liver sample arrays, a smaller percentage (2.9–3.9%) of probes were either induced or suppressed over two fold by EGCG in wild-type or Nrf2(–/–) mice at both time points. Further analyses by the software showed that 228 well-defined genes were regulated by more than two fold (162 up and 66 down) only in C57BL/6J mice, but not in Nrf2(–/–) mice at both time points by EGCG; meanwhile, 97 (84 up and 13 down) well-defined genes were regulated over two fold by EGCG regardless of genotype at both time points in the small intestine (Fig. 2B).

Quantitative Real–Time PCR Validation of Microarray Data

To verify the data generated from the microarray, 10 genes from different categories (Table I) were chosen to confirm the EGCG regulative effects by using quantitative real-time PCR analyses as described in Materials and Methods. Values for each gene were normalized by the values of corresponding GAPDH gene and the ratios of treated/vehicle were calculated. Spearman correlation was calculated and it showed that the data generated from microarray analyses are well correlated with the results obtained from quantitative real-time PCR (Fig. 3) with a correlation coefficient R^2 of 0.751, with the exception of two high-value points that drove the correlation down quite a bit.

EGCG-Regulated Nrf2-Dependent Genes in Liver and Small Intestine

Genes that were altered only in wild-type mice, but not in Nrf2(–/–) mice, by EGCG were considered EGCG-

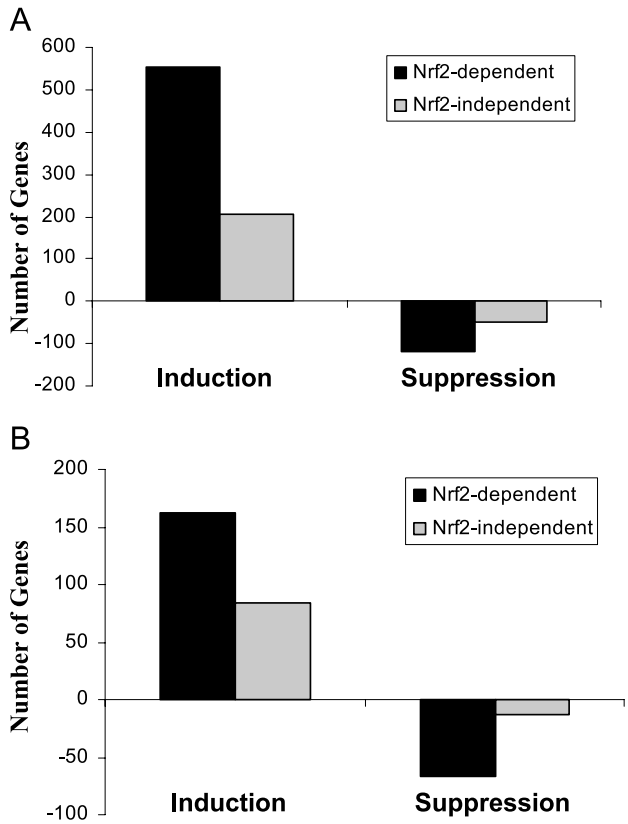

Fig. 2. Regulation of Nrf2-dependent and -independent gene expression by EGCG in mouse liver (1A) and small intestine (1B). Gene expression patterns in the liver and the small intestine were analyzed at 3 or 12 h after a single oral dose of 200 mg/kg EGCG; genes that were either induced or suppressed greater than two fold were listed. The positive number on the *y*-axis refers to the number of genes being induced; the negative number on the *y*-axis refers to number of genes being suppressed.

Table I. Oligonucleotide Primers Used in Quantitative Real-Time PCR

Gene name	GenBank	Forward primer	Reverse primer
Cytochrome *c* oxidase, subunit VIIa 2	BB745549	5′-TCTGCAGTAGGGTCCCAAGG	5′-CCAACGTTTTGCAAGCCTCT
Rho-associated coiled-coil forming kinase 2 (*ROCK2*)	BB761686	5′-TTCTGTGACCTTCAGATGGCC	5′-TTCCCAACCAGAGCACAGCT
Cytochrome P450, family 2, subfamily d, polypeptide 10 (*CYP2D10*)	BC010989	5′-TCCACTGAATTTGCCACGC	5′-TCAGCACGGAGGACATGTTG
Hemopexin (*HPXN*)	BC011246	5′-TGCGATTCAACCCTGTCACA	5′-TCTGGGTCTACCATGGCCTCT
Transporter 2, ATP-binding cassette, subfamily B (MDR/TAP) (*TAP2*)	BE691515	5′-CGTCCCTGAGCTGGTCATG	5′-GATGCTGGTGATTGCCCAC
Protein kinase C, mu	NM_008858	5′-AGCCCTTCAACGAGCAACAA	5′-ACCATCCACCCTTCCTTCATC
Inhibitor of kappaB kinase gamma (*IKBKG*)	NM_010547	5′-CTGAAAGTTGGCTGCCATGAG	5′-GAGTGGTGAGCTGGAGCAGG
APT-binding cassette, subfamily B (MDR/TAP), member 1B (*ABCB1B, MDR1*)	NM_011075	5′-GAATGTCCAGTGGCTCCGA	5′-CGGCTGTTGTCTCCATAGGC
ATPase, Cu^{2+} transporting, alpha polypeptide (*ATP7A*)	U03434	5′-TTGTGGCGGCTGGTACTTCT	5′-CAAATGCGATGGTGGTTGC
Cadherin 4 (*CDH4*)	NM_009867	5′-GACATCCCCATCCGCTACAG	5′-CGAGTGACATACATCCGGCC
Glyceraldehyde-3-phophate dehydrogenase (GAPDH)	NM_008084	5′-CACCAACTGCTTAGCCCCC	5′-TCTTCTGGGTGGCAGTGATG

regulated Nrf2-dependent genes. A selected group of these types of genes were categorized based on their biological functions, such as ubiquitination and proteolysis, electron transport, detoxification, transport, cell growth and apoptosis, cell adhesion, kinase and phosphatase, and transcription (Table II).

In the category of ubiquitination and proteolysis, liver gene expression is much more sensitive to EGCG treatment than in small intestine. In liver, EGCG induced several ubiquitination-related genes including ubiquitin fusion degradation 1-like (*UFD1L*), ubiquitin-specific protease 14 (*USP14*), and ubiquitin-conjugating enzyme E2I (*UBE2I*). Interestingly, a previous study showed that these genes were also similarly regulated in an Nrf2-dependent manner by dithiolethione (49). Another big category of genes identified were xenobiotic metabolism enzyme genes including phase I, phase II, and transporter genes. EGCG induced Nrf2-dependent genes including *CYP4A10*, catalytic subunit of glutamate-cysteine ligase (*GCLC*), gamma-glutamyltransferase 1 (*GGT1*), aldehyde reductase-like 6 (ALDRL6), sialyltransferase 10 (*ST3GAL6*) in liver, and heme oxygenase 1 (*HMOX1*, *HO-1*) in the small intestine. Interestingly, arachidonate 12-lipoxygenase (*ALOX12*), nitric oxide synthase1 (*NOS1*), and endothelial cell nitric oxide synthase 3 (*NOS3*) genes were all strongly suppressed. In the liver, EGCG induced several ATP-binding cassette family genes (*MDR1* and *TAP2*) and transporter genes involved in the H^+ (*ATP5G2*), Cu^{2+} (*ATP7A*, *ATP7B*), Cl^- (*MCLCA1*), and fatty acid (*FABP4*) transport. Many solute family member genes (*SLC4A4*, *SLC9A8*, *SLC12A4*, *SLC12A6*, *SLC13A2*, *SLC16A1*, *SLC18A2*, and *SLC37A3*) involved in transporting cellular products such as organic cation, sodium-dependent dicarboxylate, monocarboxylic acid, sodium/hydrogen, and glycerol-3-phosphate were all induced in the liver by EGCG in an Nrf2-dependent manner. As for the transporter genes in the small intestine, hemopexin (*HPXN*) and major urinary protein 3 (*MUP3*) genes, which are early response genes, their expression levels were dramatically induced by EGCG. Solute family transporter genes such as *SLC4A11*, *SLC12A9*, *SLC17A1*, *SLC35A2*, and *FPN1* were also induced by EGCG. Interestingly, the same solute carrier family gene *SLC6A14* (*ATB0,+*) was the most highly induced solute carrier family gene in both liver and small intestine, suggesting a possible dominant role for Nrf2 in EGCG-elicited regulation of this gene.

Groups of genes related to apoptosis, cell adhesion, and signaling pathways were also regulated by EGCG in both liver and small intestine. These include the induction of apoptotic protease activating factor 1 (*APAF1*) and BCL2-associated transcription factor 1 genes in the liver and inhibition of cell cycle control related p21-activated kinase 2 and 3 (*PAK2* and *PAK3*) genes in the small intestine. The cell adhesion-related gene, cadherin 4 (*CDH4*), was the most highly induced gene in this category both in liver and small intestine by EGCG. Although EGCG has been reported to regulate many signaling pathways by disturbing the phos-

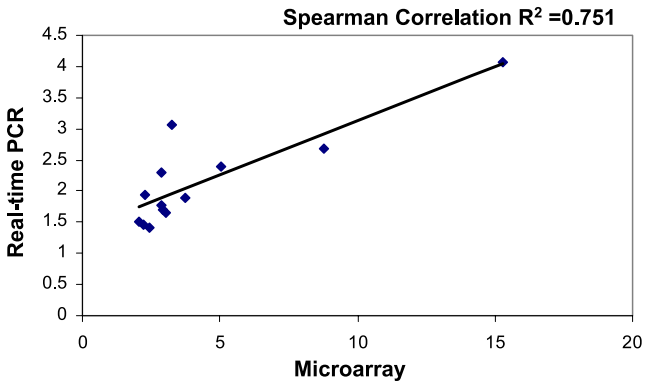

Fig. 3. Correlation of microarray data and quantitative real-time PCR data. Fold of changes in gene expression measured by real-time PCR was plotted against the corresponding fold of changes in microarray data. The Spearman correlation was calculated as R^2 = 0.751, which indicated the data from the two methods were in good correlation.

Table II. EGCG-Regulated Nrf2-Dependent Genes in Mouse Liver and Small Intestine (SIT)

			Liver[a]		SIT[b]	
Gene description	Name	GenBank	3 h	12 h	3 h	12 h
Ubiquitination and proteolysis						
A disintegrin and metalloproteinase domain 19 (meltrin beta)	*ADAM19*	NM_009616	3.67	4.86		
A disintegrin-like and metalloprotease (reprolysin type) with thrombospondin type 1 motif, 5 (aggrecanase-2)	*ADAMTS5*	BB475194	0.49	0.49		
Cathepsin M	*CSTM*	NM_022326	2.78	2.26		
Elastase 2	*ELA2*	NM_007919	0.044	6.57		
Kallikrein 5	*KLK5*	NM_008456	0.33	10.09		
Kallikrein 6	*KLK6*	BC010754	0.19	9.77		
Leishmanolysin-like (metallopeptidase M8 family)	*LMLN*	BB182358	10.08	3.40		
Matrix metalloproteinase 12	*MMP12*	BC019135	2.80	3.55		
Parkin	*PARK2*	AF250293	3.89	3.97		
Procollagen C-proteinase enhancer protein	*PCOLCE*	NM_008788	8.69	6.92		
Proliferation-associated 2G4	*PA2G4*	BM232515	2.40	2.02		
Proprotein convertase subtilisin/kexin type 5	*PCSK5*	BC013068	8.99	12.23		
Protease, serine, 2	*PRSS2*	BI348639	0.028	10.86		
Proteasome (prosome, macropain) subunit, alpha type 6	*PSMA6*	AA189256	0.47	0.49		
Proteinase 3	*PRTN3*	U97073	0.18	3.32		
Ubiquitin fusion degradation 1-like	*UFD1L*	BB500664	2.05	3.35		
Ubiquitin-specific protease 14	*USP14*	AW107924	2.97	3.32		
Ubiquitin-conjugating enzyme E2I	*UBE2I*	BM242612	3.58	2.46		
Coagulation factor IX	*F9*	M23109			17.44	3.86
Tolloid-like	*TLL1*	NM_009390			4.96	2.98
Electron transport						
Arachidonate 12-lipoxygenase	*ALOX12*	BB554189	0.20	0.45		
Cytochrome *c* oxidase, subunit VIIa 2	*COX7A2*	BB745549	3.74	2.72		
Cytochrome P450, family 2, subfamily j, polypeptide 11	*CYP2J11*	AI790773	0.42	4.63		
Cytochrome P450, family 2, subfamily j, polypeptide 13	*CYP2J13*	BC016446	0.30	3.07		
Cytochrome P450, family 2, subfamily j, polypeptide 9	*CYP2J9*	AF336850	2.13	2.32		
Cytochrome P450, family 4, subfamily a, polypeptide 10	*CYP4A1*	BC013476	2.89	4.00		
Cytochrome P450, family 4, subfamily a, polypeptide 10	*CYP4A10*	BC010747			7.20	6.15
Cytochrome P450, family 4, subfamily a, polypeptide 14	*CYP4A14*	AI327006	3.64	4.09		
Cytochrome P450, family 2, subfamily c, polypeptide 50	*CYP2C50*	NM_134144			3.93	0.25
Cytochrome P450, family 2, subfamily d, polypeptide 10	*CYP2D10*	BC010989			18.77	3.24
Nitric oxide synthase 1, neuronal	*NOS1*	AI842394	0.13	0.11		
Nitric oxide synthase 3, endothelial cell	*NOS3*	AW121498	0.34	0.25		
Phosducin	*PDC*	NM_024458	0.38	0.35		
Hydroxyacid oxidase (glycolate oxidase) 3	*HAO3*	NM_019545			6.45	3.02
Hydroxyacid oxidase 1, liver	*HAO1*	NM_010403			40.38	7.45
Thioredoxin reductase 2	*TXNRD2*	BE948556			0.35	0.21
Detoxification						
Alcohol dehydrogenase 7 (class IV), mu or sigma polypeptide	*ADH7*	NM_009626	4.52	2.77		
Aldehyde reductase (aldose reductase)-like 6	*ALDRL6*	NM_019977	2.40	3.90		
Dihydrolipoamide *S*-succinyltransferase (E2 component of 2-oxo-glutarate complex)	*DLST*	AK005477	5.76	3.67		
Sialyltransferase 10 (alpha-2,3-sialyltransferase VI)	*ST3GAL6*	NM_018784	9.60	17.26		
Gamma-glutamyltransferase 1	*GGT1*	NM_008116	2.19	5.54		
Glutamate-cysteine ligase, catalytic subunit	*GCLC*	AW825835	2.26	2.66		
Glutamate-cysteine ligase, catalytic subunit	*GCLC*	BC019374	2.60	2.36		
Aminolevulinic acid synthase 1	*ALAS1*	BM021574			5.98	3.61
Heme oxygenase (decycling) 1	*HMOX1*	NM_010442			4.16	3.07
Transport						
ATP synthase, H^+ transporting, mitochondrial F0 complex, subunit c (subunit 9), isoform 2	*ATP5G2*	AW413339	7.78	5.01		
ATPase, Cu^{2+} transporting, alpha polypeptide	*ATP7A*	U03434	2.28	2.94		
ATPase, Cu^{2+} transporting, beta polypeptide	*ATP7B*	NM_007511	2.26	8.16		
ATP-binding cassette, subfamily A (ABC1), member 5	*ABCA5*	BB128256	0.28	0.48		
ATP-binding cassette, subfamily B (MDR/TAP), member 1B	*ABCB1B*	NM_011075	6.74	5.06		
Chloride channel calcium activated 1	*MCLCA1*	AF047838	2.48	5.04		

Table II. Continued

Gene description	Name	GenBank	Liver[a] 3 h	Liver[a] 12 h	SIT[b] 3 h	SIT[b] 12 h
Transport						
Cholinergic receptor, nicotinic, alpha polypeptide 4	*CHRNA4*	BB557207	0.49	0.37		
Fatty acid binding protein 3, muscle, and heart	*FABP3*	NM_010174	0.40	0.50		
Fatty acid binding protein 4, adipocyte	*FABP4*	BC002148	2.81	2.12		
Gamma-aminobutyric acid (GABA-A) receptor, subunit alpha 1	*GABRA1*	BQ268470	0.10	0.19		
Membrane targeting (tandem) C2 domain containing 1	*TAC2-N*	AB062282	7.11	5.54		
Methyltransferase-like 1	*METTL1*	AI838750	4.52	5.34		
Myosin IC	*MYO1C*	NM_008659	2.02	2.89		
Potassium voltage-gated channel, subfamily Q, member 2	*KCNQ2*	AB000502	10.51	8.26		
RAS-like, family 2, locus 9	*RASL2-9*	NM_009028	3.11	2.85		
Solute carrier family 1 (glutamate/neutral amino acid transporter), member 4	*SLC1A4*	AI303435	0.48	0.45		
Solute carrier family 12, member 4	*SLC12A4*	NM_009195	2.68	2.07		
Solute carrier family 12, member 6	*SLC12A6*	NM_133648	2.10	2.43		
Solute carrier family 12, member 6	*SLC12A6*	AV008714	2.39	2.39		
Solute carrier family 13 (sodium-dependent dicarboxylate transporter), member 2	*SLC13A2*	BC013493	7.83	6.23		
Solute carrier family 15 (oligopeptide transporter), member 1	*SLC15A1*	NM_053079	0.35	0.43		
Solute carrier family 16 (monocarboxylic acid transporters), member 1	*SLC16A1*	NM_009196	2.15	6.35		
Solute carrier family 18 (vesicular monoamine), member 2	*SLC18A2*	BB102308	2.90	2.20		
Solute carrier family 22 (organic cation transporter), member 13	*SLC22A13*	NM_133980	0.45	0.30		
Solute carrier family 22 (organic cation transporter), member 3	*SLC22A3*	NM_011395	2.68	4.68		
Solute carrier family 24 (sodium/potassium/calcium exchanger), member 1	*SLC24A1*	BC016094	0.26	0.46		
Solute carrier family 37 (glycerol-3-phosphate transporter), member 3	*SLC37A3*	BC005744	2.53	2.98		
Solute carrier family 4 (anion exchanger), member 4	*SLC4A4*	BE655147	2.49	4.40		
Solute carrier family 6 (neurotransmitter transporter), member 14	*SLC6A14*	AF320226	30.44	13.41	10.62	3.85
Solute carrier family 7 (cationic amino acid transporter, y+ system), member 4	*SLC7A4*	BC016100	0.34	0.34		
Solute carrier family 9 (sodium/hydrogen exchanger), member 8	*SLC9A8*	AK018301	4.20	3.19		
Solute carrier organic anion transporter family, member 6d1	*OATP6D1*	AK014872	6.15	9.67		
Transporter 2, ATP-binding cassette, subfamily B (MDR/TAP)	*TAP2*	BE691515	2.43	2.56		
5-Hydroxytryptamine (serotonin) receptor 3A	*HTR3A*	NM_013561			3.30	3.24
Calcium channel, voltage-dependent, gamma subunit 6	*CACNG6*	AV091458			0.41	0.45
Calcium channel, voltage-dependent, N type, alpha 1B subunit	*CACNA1B*	AV326040			0.27	0.36
Chemokine (C–C motif) ligand 7	*SCYA7*	AF128193			8.27	8.19
Cholinergic receptor, nicotinic, alpha polypeptide 3	*CHRNA3*	BB460687			2.37	2.11
Contactin 6	*CNTN6*	NM_017383			0.07	0.48
FXYD domain-containing ion transport regulator 2	*FXYD2*	NM_052823			10.11	5.26
Gamma-aminobutyric acid (GABA-A) receptor, subunit alpha 3	*GABRA3*	NM_008067			0.42	0.44
Gamma-aminobutyric acid (GABA-A) receptor, subunit beta 3	*GABRB3*	BQ175666			0.47	0.26
Hemopexin	*HPXN*	BC011246			107.67	12.84
Major urinary protein 3	*MUP3*	M27608			447.16	29.00
Major urinary protein 3	*MUP3*	M16359			77.12	2.27
Solute carrier family 10 (sodium/bile acid cotransporter family), member 1	*SLC10A1*	BC021154			2.43	0.20
Solute carrier family 12 (potassium/chloride transporters), member 9	*SLC12A9*	BB668140			3.80	4.27
Solute carrier family 12, member 1	*SLC12A1*	NM_011389			0.41	0.23
Solute carrier family 17 (sodium phosphate), member 1	*SLC17A1*	NM_009198			2.44	2.55
Solute carrier family 35 (UDP-galactose transporter), member 2	*SLC35A2*	AU080926			2.32	2.52
Solute carrier family 4, sodium bicarbonate transporter-like, member 11	*SLC4A11*	BB498904			3.34	2.34
Solute carrier family 40 (iron-regulated transporter), member 1	*FPN1*	AF226613			2.14	3.84
Zinc finger protein 316	*ZFP316*	AV367169			3.06	2.70

Table II. Continued

Gene description	Name	GenBank	Liver[a] 3 h	Liver[a] 12 h	SIT[b] 3 h	SIT[b] 12 h
Cell growth and apoptosis						
Apoptotic protease activating factor 1	*APAF1*	AK018076	13.70	8.64		
BCL2-associated transcription factor 1		AV306063	2.16	2.25		
Bcl2-interacting killer-like	*BIKLK*	NM_007546	2.74	2.08	5.24	9.25
CCCTC-binding factor		BM199862	2.87	4.21		
Contactin 1	*CNTN1*	NM_007727	0.49	0.48		
Hepatoma-derived growth factor	*HDGF*	C80147	0.10	0.21		
p21 (CDKN1A)-activated kinase 2	*PAK2*	AK019899			0.50	0.41
p21 (CDKN1A)-activated kinase 3	*PAK3*	BB468082			0.47	0.47
RAD23b homolog (*S. cerevisiae*)	*RAD23B*	BB482313	2.80	2.14		
RAD51-like 1 (*S. cerevisiae*)	*RAD51L1*	NM_009014			3.24	2.68
Tnf receptor-associated factor 3	*CRAF1*	U21050	4.52	4.49		
Tripartite motif-containing 35	*TRIM35*	BQ175280	3.51	3.87		
Tumor differentially expressed 1	*TDE1*	NM_012032	3.66	2.20		
Catenin beta interacting protein 1	*CATNBIP1*	BF457754			0.47	2.16
Cell adhesion						
Cadherin 4	*CDH4*	NM_009867	15.26	8.80	10.24	6.38
Catenin alpha-like 1	*CATNAL1*	BQ031240	5.60	3.89		
Integrin alpha 6	*ITGA6*	BM935811	5.10	2.38		
Laminin, beta 3	*LAMB3*	NM_008484	2.96	2.31		
Neogenin		BB243938	4.15	3.51		
Neurotrimin	*HNT*	AF282980	0.33	0.36		
Osteomodulin	*OMD*	NM_012050	3.62	2.32		
Procollagen, type IV, alpha 3	*COL4A3*	AV366831	0.41	0.16		
Protocadherin 18	*PCDH18*	BM218630	3.92	3.23		
Protocadherin beta 10	*PCDHB10*	NM_053135	0.37	4.24		
Retinoschisis 1 homolog (human)	*RSIH*	NM_011302	0.28	0.12		
Cadherin 22	*CDH22*	AB019618			2.29	2.15
Cartilage link protein 1	*CRTL1*	AF098460			0.38	0.30
Procollagen, type IX, alpha 1	*COL9A1*	AK004383			3.67	3.01
Procollagen, type V, alpha 2	*COL5A2*	AV229424			2.06	4.73
Regenerating islet-derived 1	*REG1*	NM_009042			2.69	2.44
Thrombospondin 2	*THBS2*	NM_011581			2.16	3.35
Putative neuronal cell adhesion molecule	*PUNC*	BG067286			0.36	0.40
Kinase and phosphatase						
Casein kinase II, alpha 1 polypeptide	*CSNK2A1*	BB283759	3.76	3.34		
Insulin-like growth factor I receptor	*IGFIR*	BE980124	0.37	0.47	0.22	0.39
MAP/microtubule affinity-regulating kinase 1	*MARKL1*	AW491150	0.47	0.43		
Microtubule associated serine/threonine kinase 2	*MAST2*	BB367890	5.44	12.75		
Mitogen-activated protein kinase kinase 6	*MKK6*	BB540608	0.46	0.14		
Mitogen-activated protein kinase 8 interacting protein 3	*JIP3*	AF178636	3.27	3.48		
Mitogen-activated protein kinase kinase kinase kinase 4	*MAP4K4*	BQ175905	5.94	5.59		
Mitogen-activated protein kinase kinase kinase kinase 5	*MAP4K5*	BG067961	4.94	6.46		
Protein kinase C, alpha	*PRKCA*	BB355213	5.31	3.00		
Protein kinase C, mu	*PRKD1*	AV297026	4.75	0.32		
Protein kinase, AMP-activated, beta 2 noncatalytic subunit	*PRKAB2*	AV223660	16.27	18.69		
Protein kinase, cAMP-dependent regulatory, type II beta	*PRKAR2B*	BB216074	14.12	6.60		
Rho-associated coiled-coil forming kinase 1	*ROCK1*	BI662863	2.72	2.54		
Rho-associated coiled-coil forming kinase 2	*ROCK2*	BB761686	2.88	3.05		
Serine/threonine kinase 19	*STK19*	BC022681	9.30	3.53		
Serum/glucocorticoid regulated kinase 3	*SGK3*	BB768208	6.24	9.26		
Tousled-like kinase 2 (Arabidopsis)	*TLK2*	AK006771	4.70	5.48		
Tyrosine kinase receptor 1	*TIE1*	NM_011587	2.46	2.62		
Double cortin and calcium/calmodulin-dependent protein kinase-like 1	*DCAMKL1*	AW105916			0.42	0.43
Mitogen-activated protein kinase kinase kinase 10	*MAP3K10*	AA789425			10.51	11.10
Protein kinase C, mu	*PRKCM*	NM_008858			2.82	2.37
Rab38, member of RAS oncogene family	*RAB38*	NM_028238			0.44	0.34
Protein phosphatase 1A, magnesium-dependent, alpha isoform	*PPM1A*	C85630	9.55	13.78		
Protein tyrosine phosphatase, nonreceptor type 21	*PTPN21*	AW987375	8.68	7.36		
Protein tyrosine phosphatase, receptor type, E	*PTPRE*	U35368	2.98	3.25		
Protein phosphatase 1, regulatory (inhibitor) subunit 16B	*PPP1R16B*	BB375209	4.98	2.84		

Table II. Continued

Gene description	Name	GenBank	Liver[a] 3 h	Liver[a] 12 h	SIT[b] 3 h	SIT[b] 12 h
Kinase and phosphatase						
Protein phosphatase 4, regulatory subunit 1	*PPP4R1*	BC026489	2.15	2.23		
Sphingosine-1-phosphate phosphatase 1	*SGPP1*	NM_030750	2.22	2.42		
Transcription						
Ankyrin repeat and SOCS box-containing protein 3	*IGF1R*	BB002295	0.13	0.47		
Ankyrin repeat domain 6	*ANKRD6*	BM199504	0.34	0.35		
Basic transcription element binding protein 1	*BTEB1*	NM_010638	3.75	2.96		
Basic transcription element binding protein 1	*BTEB1*	AI267126	2.17	2.88		
BRAF35/HDAC2 complex		BB448266	5.43	3.84		
cAMP responsive element modulator	*CREM*	AU258667	5.11	5.09		
CREB binding protein	*CREBBP*	BG076163	7.31	2.96		
E4F transcription factor 1	*E4F1*	NM_007893	3.94	2.02		
Ewing sarcoma homolog	*EWSR1*	AW610680	11.84	9.75		
Forkhead box P1	*FOXP1*	BG962849	2.61	2.69		
Hairy/enhancer-of-split related with YRPW motif 2	*HEY2*	NM_013904	0.17	0.13		
Heterogeneous nuclear ribonucleoprotein A/B	*HNRPAB*	AK013709	7.63	7.39		
Histone deacetylase 8	*HDAC8*	AK011332	2.68	3.23		
Homeodomain leucine zipper-encoding gene	*HOMEZ*	AV298304	10.20	9.25		
Inhibitor of kappaB kinase gamma	*IKBKG*	BB147462	2.01	2.71		
Inhibitor of kappaB kinase gamma	*IKBKG*	NM_010547	2.04	2.23		
Iroquois related homeobox 5 (Drosophila)	*IRX5*	NM_018826	0.48	0.38		
Kelch repeat and BTB (POZ) domain containing 10	*KBTBD10*	W09692	2.15	2.24		
Kruppel-like factor 5	*KLF5*	BC006646	0.49	0.40		
Kruppel-like factor 7 (ubiquitous)	*KLF7*	BB524597	2.74	2.80		
LIM homeobox protein 9	*LHX9*	AK013209	7.86	6.63		
Longevity assurance homolog 4 (*S. cerevisiae*)	*LASS4*	BB006809	6.08	12.00		
Machado–Joseph disease	*MJD*	AI647473	2.47	2.14		
Nuclear receptor subfamily 2, group C, member 2	*NR2C2*	AU066920	3.01	2.19		
Nuclear receptor subfamily 2, group F, member 2	*NR2F2*	AI463873	3.72	2.18		
Paired-like homeobox 2a	*PHOX2A*	NM_008887	0.35	0.16		
PHD finger protein 10		AV024517	2.43	2.68		
RAR-related orphan receptor beta	*RORB*	BB751387	2.57	2.28	0.50	0.27
Regulatory factor X, 3 (influences HLA class II expression)	*RFX3*	BC017598	2.19	0.47		
Regulatory factor X, 4 (influences HLA class II expression)	*RFX4*	AV255458	2.13	2.98		
Retinoblastoma-like 2	*RBL2*	AA138720	2.16	2.08		
Retinoid X receptor gamma	*RXRG*	NM_009107	2.08	2.38		
Runt-related transcription factor 1	*RUNX1*	AB046930	0.45	0.37		
SCAN-KRAB-zinc finger gene 1	*ZPF306*	BC007473	2.60	2.71		
Sine oculis-related homeobox 1 homolog (Drosophila)	*SIX1*	BB137929	0.46	0.24		
Spi-C transcription factor (Spi-1/PU.1 related)	*SPIC*	NM_011461	2.24	0.09		
Suppressor of K^+ transport defect 3	*SKD3*	NM_009191	2.21	2.96		
SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin, subfamily a, member 3	*SMARCA3*	AF010600	3.65	4.67		
TAF5 RNA polymerase II, TATA box binding protein (TBP)-associated factor	*TAF5*	AV117817	3.82	3.91		
TAR DNA binding protein	*TARDBP*	BC012873	2.30	2.34		
T-box 3	*TBX3*	BB728182	2.78	2.65		
Transcription factor 12	*TCF12*	BB540782	2.81	2.09		
Transcription factor 4	*TCF4*	BG070069	3.30	5.85		
vh55e03.x1		AI480666	2.19	2.29		
WD repeat domain 9	*WDR9*	BM230348	2.39	2.01		
Zinc finger proliferation 1	*ZIPRO1*	AI326272	5.39	4.38		
Ankyrin repeat domain 1 (cardiac muscle)	*ANKRD1*	AK009959			7.86	10.87
cAMP responsive element binding protein 1	*CREB1*	NM_009952			0.23	0.27
CBFA2T1 identified gene homolog (human)	*CBFA2T1H*	BG072085			13.23	17.19
E4F transcription factor 1		BB027397			4.26	5.51
Germ cell-specific ankyrin, SAM and basic leucine zipper domain containing protein	*ASZ1*	NM_023729			2.84	3.74
Homeo box A4	*HOAX4*	AV206827			0.32	0.19
Kruppel-like factor 4 (gut)	*KLF4*	BG069413			2.36	2.26
Mitochondrial ribosomal protein S25	*MRPS25*	AK004037			2.23	2.14
Myeloid ecotropic viral integration site 1	*MEIS1*	AW547821			3.14	5.01

Table II. Continued

Gene description	Name	GenBank	Liver[a] 3 h	Liver[a] 12 h	SIT[b] 3 h	SIT[b] 12 h
Transcription						
Nuclear receptor subfamily 0, group B, member 2	*NR0B2*	BC019540			2.87	4.93
POU domain, class 2, transcription factor 2	*OCT2*	X57938			10.80	9.90
Peroxisome proliferator-activated receptor alpha	*PPARA*	BC016892			0.32	0.37
Pre B-cell leukemia transcription factor 2	*PBX2*	NM_017463			2.75	2.45
Sine oculis-related homeobox 4 homolog (Drosophila)	*SIX4*	AI893638			2.06	3.30
T-cell leukemia, homeobox 3	*TLX3*	NM_019916			2.76	3.17
Zinc finger protein 2	*ZFP2*	NM_009550			11.83	17.63
Zinc finger protein 37	*ZFP37*	NM_009554			5.87	3.74
Zinc finger protein 68	*ZFP68*	NM_013844			2.31	3.43

[a] Genes that were regulated by EGCG only in the liver of Nrf2 wild-type mice but not in Nrf2 knockout mice as compared to vehicle control at both time points. The relative mRNA expression levels of each gene in treatment group over vehicle group (fold of change) were listed. Number >2 means induction; number <0.5 means suppression.

[b] Genes that were regulated by EGCG only in the small intestine of Nrf2 wild-type mice but not in Nrf2 knockout mice as compared to vehicle control at both time points. The relative mRNA expression levels of each gene in treatment group over vehicle group (fold of change) were listed. Number >2 means induction; number <0.5 means suppression.

phorylation status of kinase or phosphatase, the microarray results clearly indicated that EGCG treatment could also regulate the gene expression of many kinases and phosphatases depending on the status of Nrf2. For example, insulin-like growth factor 1 receptor (*IGFIR*) gene expression was strongly suppressed both in liver and small intestine, and a member of RAS oncogene family Rab38 (*RAB38*) was inhibited in the small intestine.

A wide variety of transcription-related genes were regulated by EGCG in an Nrf2-dependent manner. The microarray data indicated that more of these genes were regulated in the liver than in the small intestine by EGCG. Genes that were induced including inhibitor of kappaB kinase gamma (*IKBKG*, *IKKγ*), CREB binding protein (*CREBBP*, *CBP*), retinoblastoma-like 2 (*RBL2*), retinoid X receptor gamma (*RXRG*, *RXRγ*), and histone deacetylase 8 (*HDAC8*). Several interesting transcription factors, such as paired-like homeobox 2a (*PHOX2A*), runt related transcription factor 1 (*RUNX1*), and peroxisome proliferators activated receptor alpha (*PPARA*, *PPARα*) genes were suppressed by EGCG in the liver.

EGCG-Regulated Nrf2-Independent Genes in Liver and Small Intestine

With the exception of those genes that were regulated only in C57BL/6J mice described above, a list of genes that were upregulated or downregulated more than two fold in both C57/BL/6J and Nrf2(–/–) mice by EGCG were also identified and classified into similar functional categories (Table III). For genes related to proteolysis, carboxypeptidases (*CPA1* and *CPB1*), elastases (*ELA2* and *ELA3B*), and protease serine 2 (*PRSS2*) genes were the most sensitive genes to EGCG treatments in the small intestine as they were induced by more than 100-fold in all the time points in both groups. It is interesting that hydroxyacid oxidase 3 (*HAO3*) and *UGT2B5* were induced considerably more in C57BL/6J mice than in Nrf2(–/–) mice, suggesting that their regulation by EGCG may be also Nrf2 genotype-dependent.

EGCG also induced several cell adhesion related genes, such as integrin alpha 8 (*ITGA8*) and procollagen type IV alpha 5 (*COL4A5*) in the liver, in an Nrf2-independent manner. Several EGCG-regulated kinases and phosphatases related to the phosphorylation of receptor-couple tyrosine kinase were identified in the liver. These include G protein-coupled receptor kinase 6 (*GRK6*), receptor-like tyrosine kinase, and protein tyrosine phosphatase receptor type G that were all induced by EGCG treatment. The double cortin and calcium/calmodulin-dependent protein kinase-like 1 gene (*DCAMKL1*) was the only gene that was suppressed by EGCG in both genotypes.

DISCUSSION

EGCG is a promising cancer chemopreventive agent and its anticancer effects have been investigated in numerous rodent carcinogenesis and tumor models. Because nuclear transcription factor Nrf2 regulates the expression of genes related to cellular defense and detoxification function, and the loss of Nrf2 function in mice results in increased susceptibility to carcinogenesis (45), it is of interest to investigate the role of Nrf2 in EGCG-elicited global gene expression profiles *in vivo*. One distinct expression pattern found in our current study is that more genes were regulated by EGCG in the liver than in the small intestine in both genotypes. Although the oral administration of EGCG could generate very high concentrations of EGCG in the intestinal tissue (50), differences in the gene expression patterns between the liver and the small intestine could be related to differences in the abundance of nuclear transcription factors and/or other signaling molecules in response to EGCG between the cells of these two tissues. For example, the Nrf2 expression level and nuclear coactivators available to interact with Nrf2 may determine in part how large the pool of its target genes could be regulated. Interestingly, Nrf2 expression level has been found to be much higher in the liver than in the small intestine in humans (51).

Table III. EGCG-Regulated Nrf2-Independent Genes in Mouse Liver and Small Intestine (SIT)

Gene description	Name	GenBank	Nrf2(+/+)[a]		Nrf2(–/–)[b]	
			3 h	12 h	3 h	12 h
Ubiquitination and proteolysis						
Liver						
A disintegrin and metalloprotease domain 11	*ADAM11*	NM_009613	8.95	5.80	7.89	4.05
Carboxypeptidase A1	*CPA1*	AK003088	0.01	12.80	0.44	2.29
Carboxypeptidase E	*CPE*	BC010197	9.13	3.23	3.37	5.52
Cathepsin G	*CTSG*	NM_007800	0.48	0.20	0.25	0.10
Protease, serine, 2	*PRSS2*	BI713841	0.04	10.36	2.67	5.22
Ubiquitin-conjugating enzyme E2D 3 (UBC4/5 homolog, yeast)	*UBE2D3*	AK009276	3.88	3.24	3.74	4.07
Small intestine						
Carboxypeptidase A1	*CPA1*	AK003088	125.89	139.11	105.67	167.18
Carboxpyeptidase B1 (tissue)	*CPB1*	AK003061	127.63	131.86	102.98	164.99
Elastase 2	*ELA2*	NM_007919	124.41	130.75	104.42	154.98
Elastase 3B, pancreatic	*ELA3B*	BI439657	138.22	145.64	116.13	210.82
Elastase 3B, pancreatic	*ELA3B*	NM_026419	135.00	140.13	109.66	197.20
Elastase 3B, pancreatic	*ELA3B*	AV060902	115.63	124.51	101.62	158.85
Elastase 3B, pancreatic	*ELA3B*	BI439550	114.49	121.70	100.37	153.72
Elastase 3B, pancreatic	*ELA3B*	NM_026419	38.14	34.48	46.66	197.20
Kallikrein 5	*KLK5*	NM_008456	16.22	17.38	26.39	30.55
Kallikrein 5	*KLK5*	NM_008456	4.88	4.56	4.17	4.03
Kallikrein 6	*KLK6*	BC010754	6.94	7.66	2.63	4.22
Kallikrein 6	*KLK6*	BC010754	5.49	6.12	2.53	4.17
Kallikrein 9	*KLK9*	M17962	6.75	7.70	2.51	3.87
Matrix metalloproteinase 24	*MT5MMP*	AB021226	7.54	4.88	2.02	0.35
Plasminogen	*PLG*	NM_008877	66.24	6.47	4.95	6.34
Protease, serine, 2	*PRSS2*	BI713841	119.52	123.00	107.22	172.38
Protease, serine, 2	*PRSS2*	BI348548	117.86	121.59	106.38	163.48
Protease, serine, 2	*PRSS2*	BI348639	117.56	121.77	102.78	165.20
Protease, serine, 2	*PRSS2*	NM_009430	117.00	119.26	102.23	149.25
Synonym: mGk-4; go_component: extracellular space	*NGFA*	NM_010915	3.67	2.67	2.83	2.51
Unnamed protein product; chymotrypsin-like	*CTRL*	AK003074	150.16	152.75	110.80	193.11
Electron transport						
Liver						
Cytochrome *c* oxidase, subunit VIIc	*COX7C*	AA190297	9.10	14.93	9.27	8.56
Hydroxyacid oxidase (glycolate oxidase) 3	*HAO3*	NM_019545	34.00	18.19	3.91	2.75
Thioredoxin reductase 3	*TXNRD3*	AF349659	2.19	3.38	3.23	2.77
Detoxification						
Liver						
Glutathione synthetase	*GSS*	AW553564	2.41	2.67	2.56	5.05
Small intestine						
Aldehyde dehydrogenase 2, mitochondrial	*ALDH2*	AI462635	0.29	0.13	3.90	0.47
Aldehyde dehydrogenase 2, mitochondrial	*ALDH2*	AI462635	0.17	0.07	2.08	0.27
Synonyms: IAP, Akp-3;	*AKP3*	NM_007432	2.58	6.85	3.60	11.96
UDP-glucuronosyltransferase 2 family, member 5	*UGT2B5*	AI118428	3.93	14.14	2.50	2.54
Transport						
Liver						
ATP-binding cassette, subfamily A (ABC1), member 13	*ABCA13*	BB503961	2.30	0.45	3.29	2.09
Calcium channel, voltage-dependent, P/Q type, alpha 1A subunit	*CACNA1A*	AB066608	12.97	3.78	5.73	5.33
Chloride channel 3	*CLCN3*	BB328803	10.29	13.17	2.08	2.18
Chloride intracellular channel 5	*CLIC5*	AA210377	2.54	2.25	3.04	2.67
Component of oligomeric golgi complex 1	*COG1*	BB210424	6.08	3.06	2.34	2.06
FXYD domain-containing ion transport regulator 2	*FXYD2*	NM_052823	3.55	0.43	2.33	2.92
Glutamate receptor, ionotropic, AMPA4 (alpha 4)	*GRIA4*	BB130399	0.47	0.44	0.42	0.49
Membrane targeting (tandem) C2 domain containing 1		BB548141	12.06	19.76	5.57	7.50
Mitochondrial folate transporter/carrier	*MFTC*	AK011759	2.27	2.01	2.35	2.22
Solute carrier family 15 (H^+/peptide transporter), member 2	*SLC15A2*	BC018335	0.40	0.36	0.41	0.46
Solute carrier family 39 (zinc transporter), member 14	*SLC39A14*	BB022806	2.33	2.20	3.30	2.12
Solute carrier family 5 (sodium/glucose cotransporter), member 1	*SLC5A1*	AV371434	4.41	2.64	0.47	0.12
Solute carrier organic anion transporter family, member 1a6	*SLCO1A6*	NM_023718	0.16	0.28	0.06	0.20
Synaptotagmin 4	*SYT4*	AV336547	0.42	0.47	0.32	0.48
Vesicle transport through interaction with t-SNAREs homolog 1A (yeast)	*VTI1A*	BC019386	4.45	5.74	2.45	2.25

Table III. Continued

Gene description	Name	GenBank	Nrf2(+/+)[a] 3 h	Nrf2(+/+)[a] 12 h	Nrf2(–/–)[b] 3 h	Nrf2(–/–)[b] 12 h
Transport						
Small intestine						
Apolipoprotein C-IV	*APOC4*	BC024657	45.41	5.88	3.07	4.93
Murinoglobulin 1	*MUG1*	NM_008645	299.33	7.71	0.29	5.13
Solute carrier organic anion transporter family, member 1b2	*SLC21A6*	AF250912	18.96	0.27	0.38	4.26
Sorting nexin 15	*SNX15*	BB538688	7.58	18.63	2.17	2.09
Cell cycle and cell adhesion						
Liver						
Cadherin 8	*CDH8*	BB426483	0.29	0.22	0.49	0.13
Integrin alpha 8	*ITGA8*	BB623587	8.52	8.11	4.28	4.84
Procollagen, type IV, alpha 5	*COL4A5*	BM250666	2.31	2.35	5.17	4.26
Protocadherin beta 15	*PCDHB15*	BB174795	0.43	0.33	0.25	0.14
Small intestine						
Vitronectin	*VTN*	NM_011707	7.04	2.14	2.59	3.91
MAS1 oncogene	*MAS1*	NM_008552	0.20	0.05	0.40	0.09
Kinase and phosphatase						
Liver						
Double cortin and calcium/calmodulin-dependent protein kinase-like 1	*DCAMKL1*	AW105916	0.43	0.47	0.37	0.31
G protein-coupled receptor kinase 6	*GRK6*	AF040748	5.16	6.77	2.88	2.55
Induced in fatty liver dystrophy 2		BB508622	5.31	5.79	2.31	2.60
Receptor-like tyrosine kinase		BG229030	2.31	2.44	2.32	2.56
Tousled-like kinase 2 (Arabidopsis)	*TLK2*	NM_011903	2.02	2.28	2.11	2.21
Wee 1 homolog (*S. pombe*)	*WEE1*	NM_009516	2.05	0.07	2.00	0.38
CDC14 cell division cycle 14 homolog A (*S. cerevisiae*)	*CDC14A*	BB479310	9.35	4.19	4.33	7.89
CDC14 cell division cycle 14 homolog A (*S. cerevisiae*)	*CDC14A*	BB151822	0.07	2.11	12.20	4.78
Dual specificity phosphatase 4	*DUSP4*	AK012530	2.73	2.33	21.00	5.46
Inositol (*myo*)-1(or 4)-monophosphatase 2	*IMPA2*	NM_053261	3.20	3.81	3.81	5.76
Protein tyrosine phosphatase, receptor type, G		AK017277	3.03	2.16	2.55	2.29
Transcription						
Liver						
Ankyrin repeat domain 10		BM293412	3.44	3.03	3.35	3.62
Ewing sarcoma homolog		BB699868	2.03	2.19	2.04	2.95
Forkhead box Q1		AV009267	3.73	0.33	0.29	0.21
General transcription factor II I repeat domain-containing 1	*GTF2IRD1*	AF343349	4.82	5.28	2.52	3.00
Homeo box C8	*HOXC8*	BB283726	15.74	14.39	0.03	0.50
Homeo box gene HB9	*HLXB9*	NM_019944	0.28	0.36	0.43	0.33
Histone cell cycle regulation defective homolog A (*S. cerevisiae*)	*HIRA*	AW537496	4.05	2.34	4.66	3.08
Histone deacetylase 6	*HDAC6*	NM_010413	5.22	6.32	2.40	2.18
Inhibitor of growth family, member 1-like	*ING1L*	NM_023503	2.37	2.44	2.40	2.62
Kruppel-like factor 5		BG069607	4.09	2.75	3.18	5.02
Nuclear receptor subfamily 1, group D, member 1	*NR1D1*	W13191	2.33	3.27	2.46	2.73
Nuclear receptor subfamily 2, group F, member 2	*NR2F2*	AI463873	2.94	2.44	5.21	2.61
Transcriptional regulator, SIN3A (yeast)		AW553200	9.07	6.79	8.68	6.83
Zinc finger protein 354C	*ZFP354C*	NM_013922	5.83	3.76	2.86	4.21
Zinc finger protein 143	*ZFP143*	NM_009281	3.73	2.73	5.39	5.77

[a] Genes that were regulated by EGCG in Nrf2 wild-type mice regardless of Nrf2 status at both time points. The relative mRNA expression levels of each gene in treatment group over vehicle group (fold of change) were listed. Number >2 means induction; number <0.5 means suppression.

[b] Genes that were regulated by EGCG in Nrf2 knockout mice regardless of Nrf2 status at both time points. The relative mRNA expression levels of each gene in treatment group over vehicle group (fold of change) were listed. Number >2 means induction; number <0.5 means suppression.

Genes that were mostly affected by the Nrf2 status were drug/xenobiotic metabolism enzymes, kinase, and tran scription factors encoding genes. In the current study, several genes belonging to *CYP2C*, *CYP2D*, *CYP2J*, and *CYP4A* families were identified as EGCG-regulated Nrf2-dependent genes for the first time. The regulation of these cytochrome P450 genes, especially *CYP2J* and *CYP4A* genes, implicated that EGCG may be involved in vascular homeostasis, such as the metabolism of fatty acid and epoxyeicosatrienoic acids (52). In agreement with the putative role of Nrf2 in regulating phase II and antioxidant enzyme genes and with the previous report (47) in which EGCG could induce ARE-mediated gene transcription, gamma-glutamyltransferase 1, *GCLC*, and heme oxygenase 1 (*HMOX1*, *HO-1*) were iden-

tified as EGCG-induced Nrf2-dependent genes. The regulation of *UGT2B5* is also considered Nrf2 genotype-dependent because it was more inducible by EGCG in C57BL/6J mice than in Nrf2(–/–) mice. Interestingly, this gene was also found to be Nrf2-dependently induced by dithiolethiones in a previous mouse microarray study (49). Another putative Nrf2 target gene cytochrome *c* oxidase, subunit VIIa (*COX7A2*) (53), was also induced by EGCG only in wild-type mouse liver. The identification of these genes strongly supported the role of Nrf2 in exerting EGCG's chemopreventive effects and validated the microarray data through biological or functional aspects. Transport-function related genes are one of the biggest gene categories regulated by EGCG in both genotypes of mice. Among these transporter genes, several ABC family transporters (such as *MDR1*) and many solute carrier family members (such as organic anion/cation transporters) were induced in the liver and/or small intestine in an Nrf2-dependent manner. Interestingly, EGCG regulates more Nrf2-dependent transporter genes than Nrf2-independent transporter genes, suggesting that Nrf2 plays a critical role in mediating EGCG-induced expression of transporter genes. It is also interesting that several transporter genes' expressions (such as *ABCA5*, *SLC7A4*, and *SLCO1A6*) were suppressed by EGCG in both types of mice. Although nuclear receptor pregnane X receptor (PXR) and constitutive androstane receptor (CAR) have been implicated in regulating the expression of numerous transporters (such as MDR, MRP and OATP) (54), the role of Nrf2 has not been fully investigated. In a recent study, Nrf2 activators butylated hydroxyanisole, oltipraz, and ethoxyquin were found to induce Mrp2-6 in C57BL/6J mouse liver, and Mrp3 induction was suggested to be mediated by Nrf2 and AhR (55). Therefore, the current study clearly suggests that Nrf2 not only mediated the transcription of phase II drug metabolism enzyme genes, but could also regulate the expression of phase III transporters. Because EGCG could regulate a wide variety of drug metabolism enzyme genes including these phase II detoxification and phase III transporter genes as indicated by the microarray data, and Nrf2 was also found to be involved in these processes, one of the potential molecular mechanisms underlying the anticarcinogenesis effects of EGCG could be the enhancement of the cellular defense system as well as the excretion or efflux of the carcinogen/metabolites by regulating Nrf2-mediated gene transcription.

Previous studies have shown that EGCG could cause cell cycle arrest and induce apoptosis in many cancer cells (23,24). From the array data, EGCG could induce *APAF1* gene by more than eight fold in the liver. The induction of Apaf-1 gene by EGCG is consistent with the recent studies (56,57) in which EGCG treatment induced the expression of Apaf-1 in breast cancer cells. P21-activated kinase 2 and 3 (PAK2 and PAK3) were suggested to be essential for Ras-induced upregulation of cyclin D1 during G_1 to S transition (58); therefore, suppression of *PAK2* and *PAK3* by EGCG may relate to its ability to cause G_1 cell cycle arrest. EGCG has been shown to inhibit cancer cell invasion and metastasis by increasing cell adhesive ability through upregulation of the beta 1 integrin subunit (26,57,59). In the microarray study, EGCG was found to induce integrin alpha 6 in the liver, and the induction of Rho-associated coiled-coil forming kinase ROCK1 and ROCK2 by EGCG may lead to enhanced integrin-mediated cell adhesion (60). As the impaired expression of cadherin genes was associated with cancer invasion and metastasis (61), the induction of cadherin genes cadherin 4 (*CDH4*) and cadherin 22 (*CDH22*) both in the liver and the small intestine by EGCG could also contribute to its cancer chemopreventive effects.

EGCG could block the activation of many signaling pathways such as VEGF, EGF, PDGF, NF-κB, ERK, and PI3K/Akt pathways as indicated by previous studies (4,32,33,36,39). Blocking these pathways is believed to play a central role in exerting the cancer chemopreventive effects of EGCG. The microarray data indicated that EGCG could regulate the expression of many kinase components related to these pathways. TNF receptor-associated factor 3 (*CRAF1*, *TRAF3*), which was induced by EGCG more than four fold, could heterodimerize with TRAF2 and inhibit the activation of NF-κB induced by TRAF2 (62), and therefore the induction of TRAF3 and IKKγ genes seems to be consistent with EGCG's inhibitory effect on NF-κB signaling pathways reported in many previous studies (38), which may be critical in EGCG's chemopreventive effects. The inhibition of insulin-like growth factor 1 receptor gene (*IGF1R*) by EGCG in both liver and small intestine is interesting because IGF-1R signaling is involved in the proliferation, invasion, and metastasis of many tumors including colorectal cancer and hepatocarcinoma by subsequently activation of ERK and PI3K/Akt pathways (63). Therefore, this is the first identification of IGF-1R as a target of EGCG *in vivo*, and the suppression of IGF-IR by EGCG in our study is consistent with a recent study showing that green tea polyphenols inhibited insulin-like growth factor I (IGF-I) signaling pathway in a prostate cancer mice model (7). The inhibition of IGF-1R gene expression was also accompanied with the suppression of Rab38 gene (*RAB38*), which is a member of RAS oncogene family in the small intestine. PKC mu (PKD1) was recently shown to phosphorylate E-cadherin, and PKD1 was downregulated in advanced human prostate cancer (64). Therefore, inducing of PKD1 in the liver at 3 h and in the small intestine by EGCG may result in stabilization of the cadherin/catenin complex. This may lead to increased cell aggregation and decreased cellular motility, contributing to inhibition of tumor metastasis. Arachidonate 12-lipoxygenase (*ALOX12*) converts arachidonic acid to 12(*S*)-hydroxyeicosatetraenoic acid (HETE), which is a signaling molecule implicated in tumor angiogenesis, growth, metastasis, and inhibition of apoptosis through the activation of NF-κB pathway (65). This enzyme and its product may also be involved in atherosclerosis and inflammation. Inhibition of this enzyme has been shown to induce apoptosis in gastric cancer cells (66). The inhibition of MAP/microtubule affinity-regulating kinase 1 (*MARKL1*) by EGCG is also interesting because overexpression of this kinase was found in many hepatic carcinoma cells and accompanied by accumulation of β-catenin (67).

Among the transcription factors that were Nrf2-dependently regulated by EGCG, retinoid X receptor gamma (RXRγ), which was induced by EGCG in our study, has been reported to induce terminal differentiation in squamous cell carcinoma lines, suggesting a potential tumor suppressor function for this transcription factor (68). The suppression of oncogenic transcription factor runt related transcription

factor (*RUNX1*) gene is important because a recent study showed that RUNX1 could interplay with DNA methyltransferases (DNMT) by forming a complex (69), and EGCG has been reported to inhibit DNMT and reactivate methylation silenced genes by demethylating the hypermethylated promoter region (70). Krupple-like factor 4 (*KLF4*) gene is highly expressed in epithelial tissues, such as the gut. Decreased or loss of *KLF4* expression has been observed in many gastric cancers (71), therefore, induction of *KLF4* by EGCG in small intestine (as shown in our study) suggested another potential mechanism of EGCG for colon cancer prevention. Peroxisome proliferator-activated receptor alpha (PPARα) is involved in fatty acid metabolism, because previous studies have shown that EGCG induced cancer cell growth inhibition and apoptosis may be associated with its ability to block fatty acid synthesis (28). The downregulation of this gene by EGCG in the small intestine is in accord with these previous findings.

For Nrf2-dependent genes that were induced by EGCG treatment, the molecular mechanisms have been well studied. It is believed that exposure of phase II detoxification enzyme inducers, including some chemopreventive agents in the cells, could result in the nuclear accumulation or phosphorylation of Nrf2 as well as its coactivators by many putative mechanisms that have been discussed previously (40). Although considerably more Nrf2-dependent genes were upregulated by EGCG in this study, many interesting genes were also suppressed by EGCG treatment in an Nrf2-dependent manner. The Nrf2-mediated downregulation of gene transcription mechanism has not been well studied. However, in a recent study, Dhakshinamoorthy *et al.* (72) discovered that overexpression of transcription factor Bach1 in HepG2 cells could negatively regulate the expression of NAD(P)H: quinone oxidoreductase 1 (NQO1) and ARE luciferase by binding to ARE as a heterodimer with small Maf proteins. Therefore, the Nrf2-dependent induction or suppression of gene expression by EGCG may not only depend on the direct effects of Nrf2, but may also depend on interactions with other transcription factors, coactivators, or corepressors of Nrf2 transcriptional activation complex in the nucleus.

Although this study focused on genes regulated by EGCG in an Nrf2-dependent manner, many EGCG-regulated genes, which are Nrf2-independent, were also identified and classified into similar categories except that the number of genes was smaller. For genes related to ubiquitination and proteolysis, clusters of carboxypeptidases, elastase 3B (*ELA3B*), and protein serine 2 genes (*PRSS2*) were the most highly induced genes by EGCG in this study. A chymotrypsin-like unnamed protein product gene was also induced more than 100-fold in the small intestine, and it is interesting to note that a previous study suggested that EGCG could selectively inhibit the chymotrypsin-like activity of proteasome (73). Therefore, further investigation will be needed to address the role of EGCG on proteasome gene expression and activity. Genes such as transporters in ABC and SLC families, cell adhesion protein, G-protein coupled receptor kinases, tyrosine kinases, and transcription factors were also interesting and their role in EGCG chemoprevention deserves further investigation.

In summary, our microarray analysis provides some novel insights into the global gene expression profiles elicited in the mouse liver and small intestine by EGCG. Among these EGCG-regulated genes, clusters of Nrf2-dependent gene were identified by comparing gene expression profiles between C57BL/6J and C57BL/6J/Nrf2(–/–) mice. Many of these genes were identified as EGCG-regulated Nrf2-dependent genes for the first time, such as many transport-related genes. The ability to regulate a wide variety of Nrf2-dependent genes related to ubiquitination, drug metabolism, cell growth and adhesion, phosphorylation, and transcription by EGCG may contribute to the overall anticarcinogenesis and/or the beneficial effects of green tea consumption. Results from this study also provide important and novel insights into the molecular mechanisms underlying EGCG's cancer chemoprevention effects as well as the role of Nrf2 in its biological functions. Future studies on other naturally occurring cancer chemopreventive agents focusing on specific molecular targets or signaling pathways identified in this study would greatly extend our current knowledge on cancer chemoprevention (34).

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