Modulation of nuclear factor E2-related factor 2–mediated gene expression in mice liver and small intestine by cancer chemopreventive agent curcumin

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Abstract

Curcumin has been shown to prevent and inhibit carcinogen-induced tumorigenesis in different organs of rodent carcinogenesis models. Our objective is to study global gene expression profiles elicited by curcumin in mouse liver and small intestine as well as to identify curcumin-regulated nuclear factor E2-related factor 2 (Nrf2)–dependent genes. Wild-type C57BL/6J and Nrf2 knockout C57BL/6J/Nrf2(−/−) mice were given a single oral dose of curcumin at 1,000 mg/kg. Liver and small intestine were collected at 3 and 12 hours after treatments. Total RNA was extracted and analyzed using Affymetrix (Santa Clara, CA) mouse genome 430 array (45K) and GeneSpring 6.1 software (Silicon Genetics, Redwood City, CA). Genes that were induced or suppressed >2-fold by curcumin treatments compared with vehicle in wild-type mice but not in knockout mice were filtered using GeneSpring software and regarded as Nrf2-dependent genes. Among those well-defined genes, 822 (664 induced and 158 suppressed) and 222 (154 induced and 68 suppressed) were curcumin-regulated Nrf2-dependent genes identified in the liver and small intestine, respectively. Based on their biological functions, these genes can be classified into the category of ubiquitination and proteolysis, electron transport, detoxification, transport, apoptosis and cell cycle control, cell adhesion, kinase and phosphatase, and transcription factor. Many phase II detoxification/antioxidant enzyme genes, which are regulated by Nrf2, are among the identified genes. The identification of curcumin-regulated Nrf2-dependent genes not only provides potential novel insights into the biological effects of curcumin on global gene expression and chemoprevention but also points to the potential role of Nrf2 in these processes. [Mol Cancer Ther 2006;5(1):39–51]

Introduction

Cancer development is believed to be a multistage process, including initiation, promotion, and progression (1, 2). In 1976, Dr. Michael B. Sporn first coined the term ''chemoprevention'' and advocated using cancer chemopreventive agents to decrease the incidence of cancer (3). Since then, many natural products isolated from food and plants have been investigated for their potential as cancer chemopreventive agent. Curcumin, a naturally occurring flavonoid present in the spice turmeric, has been shown to prevent and inhibit carcinogen-induced tumorigenesis in different organs in rodent carcinogenesis models, and its cancer chemopreventive effects in these animal models have been reviewed previously (4, 5). In addition to its cancer chemopreventive activity, curcumin is also well known for its antioxidant and anti-inflammatory properties (6, 7). Therefore, numerous studies have been carried out to elucidate the molecular mechanisms of the above effects of curcumin. Based on these studies, the potential mechanisms or molecular targets of curcumin have been extensively reviewed recently (4, 5, 8, 9). These include the regulation of a variety of signal transduction pathways [such as epidermal growth factor receptor, nuclear factor-κB, activator protein-1, β-catenin/TCF, mitogen-activated protein kinase (MAPK), and Akt pathways] as well as the expression of many oncogenes (such as c-jun, c-fos, c-myc, cyclooxygenase-2, and NOS) that are involved in the cell proliferation, differentiation, apoptosis, and angiogenesis. However, the chemopreventive mechanism of curcumin, especially *in vivo*, is still not fully elucidated because the interactions between these different signal transduction pathways in response to curcumin treatment are not fully understood.

Basic leucine zipper family transcription factor nuclear factor E2-related factor 2 (Nrf2) involves the regulation of antioxidant response element (ARE)–mediated gene transcription. Under homeostasis condition, Nrf2 is sequestered in cytoplasm by Kelch-like ECH-associated protein 1 (10). Exposure of cells to oxidative stress or ARE inducers triggers the release of Nrf2 from Kelch-like ECH-associated protein 1 and facilitate its nuclear translocation (11). The

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nuclear translocation of Nrf2 and subsequent dimerization with small Maf protein and other coactivators, such as CBP, will drive the transcription of its target genes (12). One large group of these target genes is the phase II detoxification and antioxidant genes. By inducing these genes through the Nrf2/ARE pathway, chemopreventive agents could increase the detoxification of procarcinogens or carcinogens and protect normal cells from the DNA/protein damage caused by electrophiles and reactive oxygen intermediates, thus decreasing the incidence of tumor initiation and reducing the risk of cancer. The role of Nrf2 in preventing tumorigenesis is also supported by studies in which Nrf2 knockout mice were much more susceptible to carcinogen-induced carcinogenesis and failed to respond to certain cancer chemopreventive agents, which were effective in Nrf2 wild-type mice (13–15). Therefore, Nrf2 has been considered as a molecular target of cancer chemoprevention (16). Previous studies have shown that chemopreventive agent sulforaphane and 3H-1,2-dithiole-3-thione could regulate a variety of genes, including phase II genes, in a Nrf2-dependent manner (17, 18). Curcumin has also been shown to be able to induce many phase II genes as well as ARE reporter gene activities (19). Therefore, studies investigating the role of Nrf2 in curcumin-regulated gene expression may help to identify new molecular mechanisms of the cancer protective effects of curcumin. Furthermore, it will also address other possible roles of Nrf2 in cancer chemoprevention in addition to the regulation of phase II detoxification enzyme and antioxidant enzyme genes.

Gene expression profiling using genome-based Affymetrix (Santa Clara, CA) microarray is an unbiased method to identify novel molecular targets of curcumin *in vivo*. In the current study, the global gene expression profiles elicited by oral administration of curcumin in wild-type and Nrf2-knockout C57BL/6J mice were compared by microarray analysis. The identification of curcumin-regulated Nrf2-dependent genes will yield valuable insights into the role of Nrf2 in the curcumin-mediated gene regulation and its cancer chemopreventive effects. The current study is also the first to investigate the global gene expression profiles elicited by curcumin in an *in vivo* mouse model where the role of Nrf2 is also examined.

Materials and Methods

Animal and Treatment

Nrf2 knockout mice Nrf2(−/−) (C57BL/SV129) were described previously (20). Nrf2(−/−) mice were back-crossed with C57BL/6J mice (The Jackson Laboratory, Bar Harbor, ME). Mice were genotyped for Nrf2 status by PCR amplification of genomic DNA extracted from tail. PCR amplification was carried out by using primers (3′-primer, 5′-GGAATGGAAAATAGCTCCTGCC-3′; 5′-primer, 5′-GCCTGAGAGCTGTAGGCCC-3′; and lacZ primer, 5′-GGGTTTTCCCAGTCACGAC-3′). Male C57BL/6J/Nrf2(−/−) mice from third generation of back-crossing were used in this study. Age-matched male C57BL/6J mice were purchased from The Jackson Laboratory. Mice 9 to 12 weeks old were used and housed at Rutgers Animal Facility. Mice were fed AIN-76A diet (Research Diets, Inc., New Brunswick, NJ) with free access to water *ad libitum* under 12-hour light/dark cycles. After 1 week of acclimatization, mice were treated with curcumin (Sigma, St. Louis, MO) at a dose of 1,000 mg/kg (dissolved in 50% polyethylene glycol 400 solution at concentration of 100 mg/mL) by oral gavages. The control groups were given vehicle only (50% polyethylene glycol 400 solution). Each treatment was administrated to a group of four animals for both C57BL/6J and C57BL/6J/Nrf2(−/−) mice. Mice were sacrificed 3 and 12 hours after curcumin treatment or 3 hours after vehicle treatment (control group; Fig. 1). Livers and small intestines were removed and stored in RNA Later (Ambion, Austin, TX) solution immediately.

RNA Extraction, Microarray Hybridization, and Data Analysis

Total RNA from liver and small intestine were isolated by using a method of Trizol (Invitrogen, Carlsbad, CA)

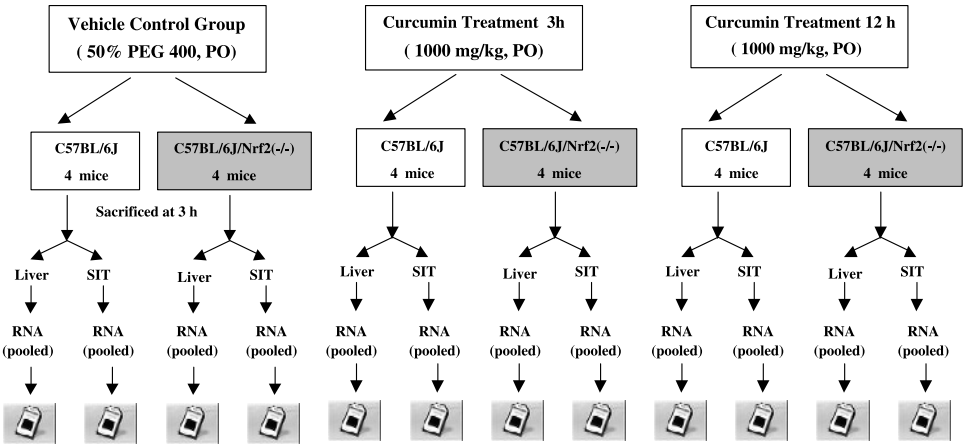

Figure 1. Schematic diagram of experimental design.

Table 1. Oligonucleotide primers used in quantitative real-time PCR

Gene name	Genbank	Forward primer (5′-3′)	Reverse primer (5′-3′)
Rho-associated coiled-coil forming kinase 2 (*ROCK2*)	BB761686	TTCTGTGACCTTCAGATGGCC	TTCCCAACCAGAGCACAGCT
PKC, μ (*PKCm*)	NM_008858	AGCCCTTCAACGAGCAACAA	ACCATCCACCCTTCCTTCATC
Inhibitor of κB kinase γ (*IKKg*)	NM_010547	CTGAAAGTTGGCTGCCATGAG	GAGTGGTGAGCTGGAGCAGG
GST, μ (*GSTm*)	NM_010358	GAAGCCAGTGGCTGAATGAGA	GATGGCATTGCTCTGGGTG
ATPase, Cu^{2+} transporting, α-polypeptide (*ATP7A*)	U03434	TTGTGGCGGCTGGTACTTCT	CAAATGCGATGGTGGTTGC
Heme oxygenase 1 (*HO-1*)	NM_010442	CCCACCAAGTTCAAACAGCTC	AGGAAGGCGGTCTTAGCCTC
Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*)	NM_008084	CACCAACTGCTTAGCCCCC	TCTTCTGGGTGGCAGTGATG

extraction coupled with the RNeasy Midi kit from Qiagen (Valencia, CA) according to the manufacturer's protocol. After RNA isolation, all the subsequent technical procedures, including quality control, concentration measurement of RNA, cDNA synthesis, and biotin labeling of cRNA, hybridization, and scanning of the arrays, were done at CINJ Core Expression Array Facility of Robert Wood Johnson Medical School (New Brunswick, NJ). Affymetrix mouse genome 430 2.0 array containing >45,101 probe sets was used to probe the global gene expression profile in mice following curcumin treatment. Each array 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 (total 12 chips were used in this study; Fig. 1). After hybridization and washing, the intensity of the fluorescence of the array chips were measured by the Affymetrix GeneChip Scanner. The expression analysis file created from each sample (chip) scanning was imported into GeneSpring 6.1 software (Silicon Genetics, Redwood City, CA) for further data characterization. A new experiment was generated after importing data from the same organ in which data were normalized to the 50th percentile of all measurements on that array. Data filtration based on flags present in at least one of the samples was generated. Lists of genes that were either induced or suppressed >2-fold between treated and 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 >2-fold only in C57BL/6J mice in both liver and small intestine were created.

Quantitative Real-time PCR for Microarray Data Validation

To verify the microarray data, several genes (including the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase) from different categories were chosen for quantitative real-time PCR analyses. The specific primers for these genes were listed in Table 1. Instead of using pooled RNA from each group, RNA samples isolated from individual mice as described above were used in real-time PCR analyses. First-strand cDNA was synthesized using 4 μg total RNA following the protocol of SuperScript III First-Strand cDNA Synthesis System (Invitrogen). Real-time PCR was done as described previously (21). The gene expression was determined by normalization with control gene glyceraldehyde-3-phosphate dehydrogenase. The correlation between corresponding microarray data and real-time PCR data was validated by Spearman rank correlation method.

Results

Curcumin-Altered Gene Expression Pattern in Mouse Liver and Small Intestine

Genes that were only regulated by curcumin in C57BL/6J mice but not in C57BL/6J/Nrf2(−/−) mice were regarded as curcumin-regulated Nrf2-dependent genes. Among these Nrf2-dependent genes, expression levels of 822 well-defined genes were either induced (664) or suppressed (158) >2-fold by curcumin only in wild-type mice liver at both time points (Fig. 2). Similar changes in gene expression profiles were also observed in the small intestine array data analysis. Compared with the results from liver sample arrays, an even smaller percentage of total probes on the array were either induced or suppressed >2-fold by curcumin regardless of Nrf2 status at both time points. Further analyses showed that 222 well-defined genes were regulated >2-fold (154 up-regulated and 68 down-regulated) in a Nrf2-dependent manner at both time points by curcumin (Fig. 2).

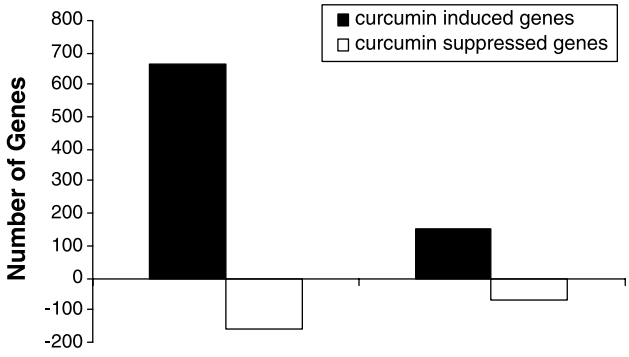

Figure 2. Regulation of Nrf2-dependent gene expression by curcumin in mice liver and small intestine. Gene expression patterns in liver and small intestine were analyzed at 3 and 12 h after a single oral dose of curcumin at 1,000 mg/kg; Nrf2-dependent genes with well known functions and regulated >2-fold at both time points were selected. The positive numbers on the *Y axis* refer to the number of genes being induced; the negative numbers on the *Y axis* refer to the number of genes being suppressed.

Curcumin-Induced Nrf2-Dependent Genes in Liver and Small Intestine

Genes that were induced only in wild-type mice but not in Nrf2(−/−) mice by curcumin were considered curcumin-induced Nrf2-dependent genes. Based on their biological functions, these genes can be classified into categories, including heat shock protein, ubiquitination and proteolysis, electron transport, detoxification enzyme, transport, cell cycle control and apoptosis, cell adhesion, kinase and phosphatase, transcription, G protein-coupled receptor, and nuclear receptor (Table 2). Among these genes, a group of curcumin-induced Nrf2-dependent phase II detoxification and antioxidant genes was identified in both liver and small intestine microarray analysis. These include *quinone reductase, catalytic subunit of glutamate-cysteine ligase* (*γ-GCS*), and *thioredoxin reductase 1* genes in liver and different isoforms of *glutathione S-transferase* (*GST*), *heme oxygenase 1* (*HO-1*), and *UDP-glucuronosyltransferase 2b5* genes in small intestine.

Surprisingly, curcumin treatment induced more genes that were not known previously as Nrf2/ARE pathway target genes than those related to Nrf2/ARE pathway in a Nrf2-dependent manner. For example, cytochrome *P*450 genes *cyp4a10* and *cyp2c55* were selectively induced in liver and small intestine, respectively. Many ubiquitination (*Usp30* and *Usp38*) and proteolysis-related (*Psmc4, Psmd9, Psme3,* etc.) genes were also induced by curcumin in a Nrf2-dependent manner, especially in liver. Another major category of genes induced by curcumin in a Nrf2-dependent manner were transporter genes. Solute carrier family member genes were the major genes to be induced in both liver and small intestine. Interestingly, several ATP-binding cassette family transporter genes, such as *Abcb1a, Abcb1b* [*multidrug resistance 1* (*MDR1*)], *Abcd3,* and *Tap2* (*Abcb3*), also seem to be Nrf2 dependently induced by curcumin in liver. Transporter genes with function of transporting ions of Cu^{2+}, K^+, Cl, and H^+ were also identified as Nrf2-dependent genes in liver. In addition to genes related to xenobiotic metabolism and excretion, genes involved in cell apoptosis, cell cycle control, cell adhesion, and signal transduction (kinase, phosphatase, and G protein-coupled receptor) were identified as targets of curcumin through Nrf2-dependent pathway. Representative genes affected in these categories include *apoptotic protease-activating factor 1, cyclin-dependent kinase inhibitor 1A* (*p21*), *cadherin* (*Cdh4, Cdh11,* and *Cdh22*), *MAPK* (*Map3k12, Map4k4,* and *Map4k5*), and *G protein-coupled receptor 65,* however, were mostly in liver. Curcumin treatment could also modulate many transcription-related genes in a Nrf2-dependent manner. These include *cyclic AMP* (*cAMP*)*–responsive element modulator, cAMP-responsive element–binding protein–binding protein, inhibitor of κB kinase γ,* and many zinc finger protein genes.

Curcumin-Suppressed Nrf2-Dependent Genes in Liver and Small Intestine

As shown in Table 3, curcumin treatment also inhibited the expression of many genes falling into similar functional categories in a Nrf2-dependent manner, although the number of genes was much smaller. *Arachidonate 12-lipoxygenase* gene was suppressed >2-fold by curcumin in liver. *Cyp11a1* and *Cyp2c50* genes were selectively inhibited in liver and small intestine, respectively. Solute carrier family genes were still the major ones in the category of transport to be suppressed in both liver and small intestine. In liver, transcription factor genes were another major category of genes being suppressed, such as forkhead box genes (*Foxf2* and *Foxm1*), homeobox genes (*Hoxb8* and *Msx2*), and Kruppel-like factor genes (*Klf3* and *Klf5*).

Quantitative Real-time PCR Validation of Microarray Data

To verify the data generated from the microarray, seven genes from different categories (Table 1) were chosen to confirm the curcumin regulation 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 glyceraldehyde-3-phosphate dehydrogenase gene and the ratios of treated/vehicle were calculated. The 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 ($R^2 = 0.74$; Fig. 3).

Discussion

The major goal of this study is to identify cancer chemopreventive agent curcumin-regulated Nrf2-dependent genes in mice liver and small intestine by using Nrf2 wild-type/knockout mice and genome-scale microarray analysis. As a cancer chemopreventive agent, curcumin could function as a cancer-blocking agent to block the tumorigenesis process in many rodent carcinogenesis models (22–24) by inducing phase II detoxification and antioxidant genes to enhance the elimination of carcinogen or reactive intermediates. During this process, Nrf2 is believed to play a central role because phase II detoxification and antioxidant genes are mainly regulated by Nrf2/ARE pathway in response to phase II inducer or chemopreventive agents. Because it is known that curcumin can induce several phase II detoxification enzyme genes and Nrf2 is critical in phase II gene induction and cancer chemoprevention, the identification of many phase II detoxification and antioxidant genes as curcumin-induced Nrf2-dependent genes in this study not only is consistent with previous studies (17, 19) but also validated the results from a biological perspective. For example, the induction of selective isoform of *GST* and oxidative-stress response gene *HO-1* is consistent with previous findings in which curcumin could induce the expression of *GST* (25, 26) and *HO-1* (19) through Nrf2/ARE pathway. The induction of *cytochrome c oxidase subunits* (*Cox7a2* and *Cox8b*) and *thioredoxin reductase 1* further supports the role of Nrf2 in curcumin-elicited gene expression because their promoter regions all contain putative Nrf2-binding sites.

Interestingly, many genes involved in phase I drug metabolism and phase III drug transporting process were also regulated by curcumin depending on Nrf2 status.

Table 2. Curcumin-induced Nrf2-dependent gene lists in liver and small intestine

Gene description	Symbol	Genbank	Liver*		Small intestine †	
			3 h	12 h	3 h	12 h
Heat shock protein						
Heat shock protein 2	Hspb2	AK012780	3.39	4.71		
Heat shock protein 1A	Hspa1a	M12573	8.28	6.24	5.88	2.22
Heat shock protein 1A	Hspa1a	M12573	6.15	4.30	5.48	2.12
Crystallin, αB	Cryab	NM_009964	6.02	6.68		
Ubiquitination and proteolysis						
A disintegrin and metalloproteinase domain 19 (meltrin β)	Adam19	NM_009616	2.67	2.68		
Cathepsin M	Cstm	NM_022326	3.68	2.14		
Dipeptidylpeptidase 9	Dpp9	BB667346	2.15	4.83		
Leishmanolysin-like (metallopeptidase M8 family)	Lmln	BB182358	5.17	5.70		
Mucosa-associated lymphoid tissue lymphoma translocation gene 1	Malt1	BM239348	2.41	2.62		
Procollagen C-proteinase enhancer protein	Pcolce	NM_008788	8.12	10.07		
Proprotein convertase subtilisin/kexin type 5	Pcsk5	BC013068	11.83	18.73		
Proteaseome (prosome, macropain) 28S subunit, 3	Psme3	U60330	2.30	2.88		
Proteasome (prosome, macropain) 26S subunit, ATPase, 6	Psmc6	AW208944	2.31	3.02		
Proteasome (prosome, macropain) 26S subunit, non-ATPase, 11	Psmd11	AA050796	2.67	3.41		
Proteasome (prosome, macropain) 26S subunit, ATPase, 4	Psmc4	NM_011874	2.52	2.60		
Proteasome (prosome, macropain) 26S subunit, non-ATPase, 9	Psmd9	BG092381	2.39	2.75		
Ring finger protein 11	Rnf11	BI150320	2.12	2.24		
Ubiquitin fusion degradation 1 like		BB500664	3.28	2.72		
Ubiquitin-specific protease 30	Usp30	BG067690	3.37	3.93		
Ubiquitin-specific protease 38	Usp38	BG064874	2.43	2.70		
Ubiquitin-conjugating enzyme E2B		AK011961	2.31	2.13		
Coagulation factor IX	F9	M23109			5.06	8.86
Proteasome (prosome, macropain) activator, subunit 4		BB200981			8.21	3.24
Tolloid-like	Tll1	NM_009390			7.80	3.61
Transferrin receptor 2	Trfr2	AV027486			3.86	4.20
Electron transport						
Aldehyde oxidase 1	Aox1	NM_009676	2.01	2.47		
Cytochrome *c* oxidase, subunit VIIa 2	Cox7a2	BB745549	4.32	4.82		
Cytochrome *c* oxidase, subunit VIIIb	Cox8b	NM_007751	3.14	2.38		
Similar to cytochrome *P*450, 4a10	Cyp4a10	BC025936	2.02	2.42		
Thioredoxin reductase 1	Txnrd1	NM_015762	2.12	2.16		
Ubiquinol-cytochrome *c* reductase core protein 1		BG864756	2.08	2.57		
Cytochrome *P*450, family 2, subfamily c, polypeptide 55	Cyp2c55	NM_028089			2.89	4.95
Detoxification enzyme						
Crystallin, ζ (quinone reductase) like 1	Cryzl1	AK010433	2.02	2.26		
Fucosyltransferase 8	Fut8	NM_016893	10.78	10.62		
Glutamate-cysteine ligase, catalytic subunit	Gclc	AW825835	2.41	4.26		
Methyltransferase-like 1	Mettl1	AI838750	4.28	5.76		
Sialyltransferase 10	Siat10	NM_018784	15.70	17.79		
Steroid sulfatase	Sts	NM_009293	2.49	3.33		
Thioredoxin interacting protein	Txnip	AF173681	2.94	2.34		
Carbonyl reductase 3	Cbr3	AK003232			21.68	13.22
GST, μ1	Gstm1	NM_010358			4.42	11.65
GST, μ1	Gstm1	J03952			2.84	7.90
GST, μ3	Gstm3	J03953			3.72	5.91
GST, α2 (Yc2)	Gsta2	NM_008182			2.79	9.58
GST, α3	Gsta3	AI172943			4.49	2.62
GST, α4	Gsta4	NM_010357			3.76	2.49

*Genes that were induced >2-fold by curcumin only in liver of Nrf2 wild-type mice but not in liver of Nrf2 knockout mice comparing with vehicle treatment at both time points. The relative mRNA expression levels of each gene in treatment group over vehicle group (fold of change) were listed.
†Genes that were induced >2-fold by curcumin only in small intestine of Nrf2 wild-type mice but not in small intestine of Nrf2 knockout mice comparing with vehicle treatment at both time points. The relative mRNA expression levels of each gene in treatment group over vehicle group (fold of change) were listed.

(*Continued on the following page*)

Table 2. Curcumin-induced Nrf2-dependent gene lists in liver and small intestine (Cont'd)

Gene description	Symbol	Genbank	Liver*		Small intestine †	
			3 h	12 h	3 h	12 h
HO-1 (decycling)	Hmox1	NM_010442			76.06	4.54
UDP-glucuronosyltransferase 2 family, member 5	Ugt2b5	NM_009467			2.07	6.70
Transport						
Aquaporin 7	Aqp7	AB056091	2.38	3.30		
ATP synthase, H^+ transporting, mitochondrial F0 complex, subunit c (subunit 9), isoform 2	Atp5g2	AW413339	4.03	5.12		
ATPase, Ca^{2+} transporting, cardiac muscle, slow twitch 2	Atp2a2	AA245637	2.12	3.10		
ATPase, class II, type 9A	Atp9a	AF011336	2.14	2.60		
ATPase, Cu^{2+} transporting, α-polypeptide	Atp7a	U03434	4.06	3.10		
ATP-binding cassette, subfamily B (MDR/TAP), member 1A	Abcb1a	M30697	2.23	2.05		
ATP-binding cassette, subfamily B (MDR/TAP), member 1B	Abcb1b	NM_011075	7.06	5.18		
ATP-binding cassette, subfamily D (ALD), member 3	Abcd3	BB042134	2.10	2.37		
Cation channel, sperm associated 2	Catsper2	BB484902	2.18	2.15		
Chloride channel 3	Clcn3	BB328803	12.31	15.49		
Chloride channel calcium activated 1	Clca1	AF047838	7.22	3.48		
Fatty acid–binding protein 4, adipocyte	Fabp4	BC002148	5.62	3.24		
FXYD domain-containing ion transport regulator 2	Fxyd2	NM_052823	3.62	11.19	4.50	6.00
Kinesin family member 5B	Kif5b	BI328541	2.27	2.41		
Membrane targeting (tandem) C2 domain containing 1	Mtac2d1	AB062282	8.52	7.72		
Myosin IC	Myo1c	NM_008659	3.28	2.79		
N-ethylmaleimide-sensitive fusion protein	Nsf	BB400581	2.20	2.95		
Potassium voltage-gated channel, Shab-related subfamily, member 1	Kcnb1	BB324482	6.09	3.17		
Potassium voltage-gated channel, subfamily Q, member 2	Kcnq2	AB000502	11.48	2.10		
Solute carrier family 12, member 2	Slc12a2	BG069505	2.27	2.71		
Solute carrier family 13 (sodium-dependent dicarboxylate transporter), member 2	Slc13a2	BC013493	5.37	4.68		
Solute carrier family 16 (monocarboxylic acid transporters), member 1	Slc16a1	NM_009196	2.25	8.09		
Solute carrier family 18 (vesicular monoamine), member 2	Pdzk8	BB102308	3.38	3.12		
Solute carrier family 2 (facilitated glucose transporter), member 9		BE197100	2.73	2.58		
Solute carrier family 22 (organic cation transporter), member 3	Slc22a3	NM_011395	3.51	5.57		
Solute carrier family 22 (organic cation transporter), member 5	Slc22a5	NM_011396	2.18	3.76		
Solute carrier family 25 (mitochondrial carrier, palmitoylcarnitine transporter), member 29	Slc25a29	BC006711	2.06	2.20		
Solute carrier family 37 (glycerol-3-phosphate transporter), member 3	Slc37a3	BC005744	2.09	2.49		
Solute carrier family 39 (zinc transporter), member 14	Slc39a14	BB022806	3.78	2.11		
Solute carrier family 6 (neurotransmitter transporter), member 14	Slc6a14	AF320226	23.50	19.96		
Solute carrier family 9 (sodium/hydrogen exchanger), member 8	Slc9a8	AK018301	2.09	4.70		
Src activating and signaling molecule	Srcasm	BC004710	2.08	2.04		
Syntaxin 6	Stx6	BB492711	2.78	3.83		
Transporter 2, ATP-binding cassette, subfamily B (MDR/TAP)	Tap2	BE691515	2.90	2.12		
ATPase, class V, type 10A	Atp10a	BM249532			5.13	3.11
Chemokine (C-C motif) ligand 7	Ccl7	AF128193			8.15	12.09
Hemopexin	Hpxn	BC011246			7.84	32.11
5-Hydroxytryptamine (serotonin) receptor 3A	Htr3a	NM_013561			2.74	2.31
Major urinary protein 3	Mup3	M27608			13.25	102.59
Solute carrier family 17 (sodium phosphate), member 1	Slc17a1	NM_009198			2.01	3.00
Solute carrier family 34 (sodium phosphate), member 2	Slc34a2	NM_011402			2.13	5.54
Solute carrier family 35 (UDP galactose transporter), member 2		AU080926			2.14	2.61
Solute carrier family 40 (iron-regulated transporter), member 1	Slc40a1	AF226613			2.22	2.43
Solute carrier family 6 (neurotransmitter transporter), member 14	Slc6a14	AF320226			3.50	3.29
Zinc finger protein 316	Zfp316	AV367169			2.34	2.00
Solute carrier family 4, sodium bicarbonate transporter-like, member 11	Slc4a11	BB498904			3.12	2.49
Sodium channel, voltage-gated, type IX, α-polypeptide		BB452274			2.72	4.16
Apoptosis and cell cycle control						
Apoptotic protease-activating factor 1	Apaf1	AK018076	16.54	14.58		
Baculoviral IAP repeat-containing 1a	Birc1a	AF135491	3.02	4.02		

(*Continued on the following page*)

Table 2. Curcumin-induced Nrf2-dependent gene lists in liver and small intestine (Cont'd)

Gene description	Symbol	Genbank	Liver*		Small intestine †	
			3 h	12 h	3 h	12 h
Bcl-2-associated transcription factor 1	Bclaf1	BI965039	2.23	2.62		
Bcl-2-like	Bcl2l1	NM_009743	2.98	3.08		
Cyclin-dependent kinase inhibitor 1A (p21)	Cdkn1a	AK007630	2.02	10.06		
Cytotoxic granule-associated RNA-binding protein 1	Tia1	BG518542	4.18	4.22		
Ring finger protein 7	Rnf7	AV047821	2.62	3.74		
Tumor necrosis factor receptor-associated factor 3	Traf3	U21050	5.78	4.25		
Transformation-related protein 53-inducible nuclear protein 1	Trp53inp1	AW495711	2.26	2.41		
Tripartite motif-containing 35		BQ175280	3.80	3.27		
Tumor differentially expressed 1	Tde1	NM_012032	3.15	4.29		
CWF19-like 2, cell cycle control (*Schizosaccharomyces pombe*)	Cwf19l2	AK014327	3.18	3.97		
Bcl-2-interacting killer-like	Biklk	NM_007546			3.41	9.60
RAS like, estrogen regulated, growth inhibitor	Rerg	BC026463			3.81	2.60
Cell adhesion						
Cadherin 11	Cdh11	NM_009866	3.54	3.35		
Cadherin 22	Cdh22	AB019618	2.00	2.54	2.37	2.77
Cadherin 4	Cdh4	NM_009867	15.48	10.96		
Catenin α-like 1	Catnal1	BQ031240	4.49	4.74		
Catenin src	Catns	NM_007615	2.15	3.08		
Contactin-associated protein 1	Cntnap1	NM_016782	4.56	4.75		
Integrin α_8	Itga8	BB623587	6.71	6.44		
Laminin, β3	Lamb3	NM_008484	2.41	5.53		
Neurotrimin		AF282980	0.49	0.43		
Osteomodulin	Omd	NM_012050	3.11	3.01		
Protocadherin 18	Pcdh18	BM218630	2.83	3.78		
Fibronectin leucine-rich transmembrane protein 2	Flrt2	BB817332			11.38	5.14
Procollagen, type IX, α1	Col9a1	AK004383			3.33	2.32
Thrombospondin 2	Thbs2	BB233297			2.06	2.51
Kinase and phosphatase						
Casein kinase II, α1 polypeptide	Csnk2a1	BB283759	3.85	2.07		
Induced in fatty liver dystrophy 2		BB508622	3.68	5.71		
Keratin complex 2, basic, gene 8	Krt2-8	AW322280	2.49	2.56		
MAPK-activated protein kinase 2	Mapkapk2	BG918951	2.07	2.84		
Microtubule-associated serine/threonine kinase 2	Mast2	BB367890	5.34	12.50		
MAPK kinase kinase 12	Map3k12	NM_009582	3.57	2.70		
MAPK 8 interacting protein 3	Mapkip8	AF178636	3.41	4.28		
MAPK kinase kinase kinase 4	Map4k4	NM_008696	3.71	4.24		
MAPK kinase kinase kinase 5	Map4k5	BG067961	8.29	7.17		
PCTAIRE-motif protein kinase 1	Pctk1	AW539955	6.66	5.81		
PKC, α		BB355213	4.49	4.04		
Protein kinase, cAMP-dependent regulatory, type IIβ	Prkar2b	BB216074	26.47	4.75		
Proviral integration site 2		NM_138606	2.00	2.45		
Regulator of G protein signaling 19	Rgs19	BC003838	2.09	2.49		
Rho-associated coiled-coil forming kinase 1	Rock1	BI662863	2.15	3.07		
Rho-associated coiled-coil forming kinase 2	Rock2	BB761686	3.07	2.54		
Ribosomal protein S6 kinase, polypeptide 5	Rps6ka5	BQ174267	2.65	2.39		
Serine/threonine kinase 17b (apoptosis inducing)	Stk17b	AV173139	3.05	2.53		
Serum/glucocorticoid-regulated kinase 3	Sgk3	BB768208	6.68	6.67		
SNF1-like kinase	Snf1lk	AI648260	7.55	9.82		
Testis-specific protein kinase 1	Tesk1	NM_011571	2.05	2.28		
Tousled-like kinase 2 (*Arabidopsis*)	Tlk2	NM_011903	2.48	2.81		
Dual specificity phosphatase 6	Dusp6	NM_026268	2.45	2.18		
Eyes absent 3 homologue (*Drosophila*)	Eya3	BB428881	2.94	3.12		
Paladin		NM_013753	2.14	2.53		
Protein tyrosine phosphatase, nonreceptor type 21	Ptpn21	AW987375	7.44	13.85		
PKC, μ	Prkcm	NM_008858			2.22	2.04
Keratin complex 2, basic, gene 8	Krt2-18	NM_016879			2.85	2.29

(*Continued on the following page*)

Table 2. Curcumin-induced Nrf2-dependent gene lists in liver and small intestine (Cont'd)

Gene description	Symbol	Genbank	Liver*		Small intestine †	
			3 h	12 h	3 h	12 h
MAPK kinase kinase 10	Map3k10	AA789425			10.02	7.37
Dual specificity phosphatase 4	Dusp4	AK012530			2.65	2.00
G protein-coupled receptors						
G protein-coupled receptor 65	Gpr65	NM_008152	6.34	5.32		
Endothelial differentiation, sphingolipid G protein-coupled receptor, 8	Edg8	NM_053190	4.92	3.92		
Endothelin receptor type A	Ednra	BC008277	9.12	10.42		
Endothelin receptor type A	Ednra	AW558570	7.84	7.60		
Transcription factors						
Ankyrin repeat domain 1 (cardiac muscle)	Ankrd1	AK009959	2.12	2.88		
Basic transcription element-binding protein 1	Bteb1	NM_010638	2.90	3.54		
cAMP-responsive element modulator	Crem	AU258667	9.73	6.29		
cAMP-responsive element modulator	Crem	AI467599	2.58	2.56		
cAMP-responsive element–binding protein–binding protein		BG076163	5.62	10.55		
cAMP-responsive element–binding protein–binding protein/ EP300 inhibitory protein 1	Cri1	BC010712	2.05	2.19		
E4F transcription factor 1	E4f1	NM_007893	3.69	3.50		
E74-like factor 1	Elf1	NM_007920	2.06	2.22		
Early growth response 1	Egr1	NM_007913	4.19	5.06		
Ewing sarcoma homologue	Ewsr1	AW610680	10.50	5.63		
Forkhead box N2	Foxn2	AV295543	3.34	2.75		
Forkhead box P1	Foxp1	BG962849	2.71	3.65		
Heterogeneous nuclear ribonucleoprotein A/B	Hnrpab	AK013709	6.55	7.28		
Heterogeneous nuclear ribonucleoprotein R	Hnrpr	BB251000	2.50	2.10		
Homeobox C8	Hoxc8	BB283726	4.83	2.57		
Homeodomain leucine zipper-encoding gene		AV298304	7.72	8.52		
Inhibitor of κB kinase γ	Ikbkg	BB147462	2.73	3.55		
Inhibitor of κB kinase γ	Ikbkg	NM_010547	2.12	2.83		
Kruppel-like factor 7 (ubiquitous)	Klf7	BB524597	2.09	2.97		
LIM homeobox protein 9	Lhx9	AK013209	3.23	5.88		
Longevity assurance homologue 4 (*Saccharomyces cerevisiae*)	Lass4	BB006809	3.18	9.26		
Nuclear factor, interleukin 3, regulated	Nfil3	AY061760	2.13	3.52		
Nuclear receptor subfamily 2, group C, member 2	Nr2c2	AU066920	2.22	2.60		
Retinoid X receptor γ	Rxrg	NM_009107	2.63	2.94		
SCAN-KRAB-zinc finger gene 1	Zpf306	BC007473	2.21	3.91		
Suppressor of K^+ transport defect 3	Skd3	NM_009191	2.80	2.76		
TAF5 RNA polymerase II, TATA box-binding protein–associated factor	Taf5	AV117817	2.96	3.90		
TAR DNA-binding protein	Tardbp	BC012873	2.63	3.96		
Transforming growth factor-β-inducible early growth response 1	Tieg1	NM_013692	2.76	3.52		
Transcription factor 12	Tcf12	BB540782	2.79	2.86		
Transcription factor 20	Tcf20	AW552808	2.22	2.14		
Transcription factor 3	Tcf3	NM_009332	4.44	5.08		
Zinc finger proliferation 1	Zipro1	AI326272	4.02	7.65		
Zinc finger protein 148	Zfp148	X98096	2.11	2.29		
Zinc finger protein 207	Zfp207	AV338324	2.16	2.11		
Zinc finger protein 263	Zfp263	AI326880	3.02	2.96		
Zinc finger protein 319	Zfp319	BB476317	5.45	5.41		
Zinc finger protein 354C	Zfp354c	NM_013922	3.33	3.73		
Zinc fingers and homeoboxes 3	Zhx3	BE952825	6.37	7.45		
Ankyrin repeat domain 1 (cardiac muscle)	Ankrd1	AK009959			3.82	16.77
CBFA2T1 identified gene homologue (human)	Cbfa2t1h	BG072085			19.43	12.24
E4F transcription factor 1		BB027397			7.90	9.35
Myeloid ecotropic viral integration site 1	Meis1	AW547821			4.31	3.67
POU domain, class 2, transcription factor 2	Pou2f2	X57938			12.27	12.47
Runt-related transcription factor 1	Runx1	NM_009821			2.24	2.64
Transforming growth factor-β1-induced transcript 4	Tgfbli4	AW413169			9.17	4.53
Zinc finger protein 2	Zfp2	NM_009550			6.84	3.90

(*Continued on the following page*)

Table 2. Curcumin-induced Nrf2-dependent gene lists in liver and small intestine (Cont'd)

Gene description	Symbol	Genbank	Liver*		Small intestine †	
			3 h	12 h	3 h	12 h
Zinc finger protein 37	Zfp37	NM_009554			7.84	5.46
Zinc finger protein 68	Zfp68	NM_013844			2.03	3.15
Others						
Amyloid-β (A4) precursor protein binding, family B, member 3	Apbb3	BC024809	2.37	2.33		
Aryl hydrocarbon receptor nuclear translocator like	Arntl	BC011080	5.12	21.26		
Breast cancer metastasis suppressor 1	Brms1	NM_134155	2.04	4.57		
Peroxisome proliferator-activated receptor–binding protein	Pparbp	NM_134027	2.56	2.63		
Suppression of tumorigenicity 7	St7	NM_022332	2.08	3.75		
Suppressor of cytokine signaling 6	Socs6	NM_018821	2.31	2.28		
Suppressor of cytokine signaling 4	Socs4	AK014988	2.72	2.09		
Tumor necrosis factor receptor-associated factor 6	Traf6	AV244412	2.22	2.14		

Cyp4a10 and *Cyp2c55* were induced in liver and small intestine, respectively, whereas *Cyp11a1* and *Cyp2c50* were suppressed; however, their roles in the cancer chemopreventive effect of curcumin remain unclear. The transport function-related genes were the major group of genes being regulated by curcumin in a Nrf2-dependent manner in both liver and small intestine. Although the interaction between curcumin and transporters, such as MDR1 and MDR-associated protein 1, has been investigated *in vitro* (27), the effects of curcumin on other transporters, especially their expression, have never been examined *in vivo*. Solute carrier family transporter genes were the major ones to be selectively regulated in liver and small intestine. Altered expression of these transporter genes could perturb the transporting of organic cation (*Slc22a3*), glycerol-3-phosphate (*Slc37a3*), and monocarboxylic acid (*Slc16a1*) and could affect sodium/hydrogen exchange (*Slc9a8*). The induction of four ATP-binding cassette transporter genes, such as *Abcb1b* (*MDR1*), suggested that Nrf2 also play a significant role in regulating ATP-binding cassette family transporter genes. Although PXR and CAR have been shown to play critical roles in regulating the expression of MDR1 and MDR-associated protein genes (28), the role of Nrf2 has not been excluded. *Hemopexin* was dramatically induced by curcumin in a Nrf2-dependent manner in small intestine. Hemopexin is critical in maintaining the homeostasis of metal ions by forming a complex with heme. As the major vehicle for the transportation of heme, hemopexin could prevent heme-mediated oxidative stress and heme-bound iron loss (29), functionally analogous to HO-1, which metabolizes heme and prevent oxidative stress. Taken together, our current study suggested that curcumin could coordinately regulate the phase I, II, and III xenobiotic metabolizing enzyme genes as well as antioxidative stress genes through Nrf2-dependent pathways *in vivo*. Such regulation (especially induction) of these genes could have significant effects on prevention of tumor initiation by enhancing the cellular defense system, preventing the activation of procarcinogens/reactive intermediates, and increasing the excretion of reactive carcinogen or metabolites.

Previous *in vitro* (30–33) and *in vivo* (34, 35) studies have suggested that curcumin could also act as a tumor-suppressing agent by regulating many cellular signal transduction pathways in cancer cells. Therefore, modulation of signaling pathways (4, 8, 9) involved in cell proliferation, cell cycle control, apoptosis, adhesion, invasion and metastasis, angiogenesis, and inflammation by curcumin were linked to its strong cancer chemopreventive effects. However, the role of Nrf2 in curcumin-elicited alternation of signaling transduction pathways related to these cellular events has never been investigated. In the current study, apoptosis-related gene *apoptotic protease-activating factor 1* was induced by curcumin >14-fold at both time points in the liver. Because the regulation of *apoptotic protease-activating factor 1* by curcumin and Nrf2 has not been reported, our results suggested that curcumin-induced cancer cell apoptosis may result from its Nrf2-dependent regulation of apoptotic protease-activating factor 1–related pathways. Cell cycle control gene *cyclin-dependent kinase inhibitor 1A* (*p21*) was induced >10-fold at 12 hours on curcumin administration. This is supported by a previous study in which curcumin cause G_1 arrest in PC-3 cells by induction of p21 (36). Curcumin has been shown to inhibit cancer cell invasion and metastasis (25, 37) by modulating integrin receptors, collagenase activity, and expression of E-cadherin. In the current study, several cadherin genes were also induced by curcumin, such as *Cdh4, Cdh11,* and *Cdh22,* in liver, although *Cdh22* gene was also induced in small intestine. The cadherin family of transmembrane glycoproteins plays a critical role in cell-to-cell adhesion, and cadherin dysregulation is strongly associated with cancer metastasis and progression (38). Because impaired expression of cadherin genes were associated with cancer invasion and metastasis (39), the induction of cadherin genes through Nrf2/ARE pathway by curcumin could be another potential mechanism of exerting its cancer chemoprevention effects. Although microarray studies cannot provide information on the regulation of kinase phosphorylation by curcumin, our results indicated that the expression of many signaling pathway members was affected in a Nrf2-dependent

Table 3. Curcumin-suppressed Nrf2-dependent gene lists in liver and small intestine

Gene description	Symbol	Genbank	Liver*		Small intestine †	
			3 h	12 h	3 h	12 h
Ubiquitination and proteolysis						
Cathepsin G	Cstg	NM_007800	0.27	0.06		
Mast cell protease 6	Mcpt6	NM_010781	0.46	0.19		
Matrix metalloproteinase 24	Mmp24	AB021226	0.24	0.35		
Kallikrein 26	Klk13	NM_010115			0.49	0.25
Carboxypeptidase A4	Cpa4	AV294399			0.19	0.36
Electron transport						
Arachidonate 12-lipoxygenase	Alox12	BB554189	0.33	0.21		
Cytochrome *P*450, family 11, subfamily a, polypeptide 1	Cyp11a1	C87524	0.38	0.37		
Interleukin-4 induced 1	Ll4i1	NM_010215	0.10	0.17		
Phosducin	Pdc	NM_024458	0.45	0.46		
Thioredoxin like 1	Txnl1	AV106191	0.33	0.20		
Cytochrome *P*450, family 2, subfamily c, polypeptide 50	Cyp2c50	NM_134144			0.14	0.09
Transport						
ATPase, aminophospholipid transporter, class I, type 8A, member 1	Atp8a1	AW610650	0.36	0.21		
ATPase, H^+ transporting, V0 subunit D, isoform 2	Atp6v0d2	AV204216	0.49	0.43		
ATP-binding cassette, subfamily C (CFTR/MDR-associated protein), member 8	Abcc8	BB515948	0.47	0.38		
Fatty acid–binding protein 3, muscle and heart	Fabp3	NM_010174	0.42	0.43		
γ-Aminobutyric acid-A receptor, subunit δ	Gabrd	NM_008072	0.50	0.37		
Glutamate receptor, ionotropic, AMPA4 (α4)	Gria4	AV336506	0.11	0.37		
Potassium channel, subfamily K, member 2	Kcnk2	NM_010607	0.41	0.23		
Potassium inwardly rectifying channel, subfamily J, member 15	Kcnj15	BB533892	0.07	0.14		
Retinaldehyde-binding protein 1	Rlbp1	NM_020599	0.27	0.06		
Structural maintenance of chromosomes 2 like 1 (yeast)	Smc2l1	BI684556	0.49	0.41		
Solute carrier family 12, member 1	Slc12a1	NM_011389	0.40	0.39		
Solute carrier family 15 (oligopeptide transporter), member 1	Slc15a1	NM_053079	0.46	0.30		
Solute carrier family 2 (facilitated glucose transporter), member 10	Slc2a10	NM_130451	0.34	0.25		
Solute carrier family 24 (sodium/potassium/calcium exchanger), member 1	Slc24a1	BC016094	0.44	0.41		
Synaptogyrin 1	Syngr1	NM_009303	0.41	0.42		
ATPase, H^+/K^+ transporting, nongastric, α-polypeptide	Atp12a	NM_138652			0.26	0.12
Glutamate receptor, ionotropic, kainate 2 (β2)		BB355480			0.23	0.36
Potassium intermediate/small conductance calcium-activated channel, subfamily N, member 2	Kcnn2	NM_080465			0.50	0.31
Solute carrier family 2 (facilitated glucose transporter), member 4	Slc2a4	AB008453			0.47	0.49
Solute carrier family 23 (nucleobase transporters), member 1	Slc23a1	AA276202			0.39	0.40
Solute carrier family 34 (sodium phosphate), member 1	Slc34a1	AK005930			0.13	0.45
Calcium channel, voltage-dependent, γ subunit 7 (Cacng7), mRNA		AF361349			0.42	0.11
Cell adhesion						
Desmocollin 1	Dsc1	NM_013504	0.07	0.24		
Neurotrimin		AF282980	0.49	0.43		
Protocadherin β16	Pcdhb16	BB131219	0.34	0.32		
Cartilage link protein 1	Hapln1	AF098460			0.09	0.32
Putative neuronal cell adhesion molecule	Punc	BG067286			0.42	0.40
Kinase and phosphatase						
Interleukin-1 receptor-associated kinase 3	Irak3	BB497580	0.43	0.42		
MAPK kinase 6		BB540608	0.50	0.30		
Protein kinase, cyclic guanosine 3′,5′-monophosphate-dependent, type II	Prkg2	BB823350	0.48	0.34		
Protein tyrosine phosphatase, receptor type, E	Ptpre	U35368	0.35	0.44		
Regulator of G protein signaling 18	Rgs18	BB139986	0.48	0.25		
Tousled-like kinase 1	Tlk1	BM244995	0.35	0.32		

*Genes that were suppressed >2-fold by curcumin only in liver of Nrf2 wild-type mice but not in liver of Nrf2 knockout mice comparing with vehicle treatment at both time points. The relative mRNA expression levels of each gene in treatment group over vehicle group (fold of change) were listed.

†Genes that were suppressed >2-fold by curcumin only in small intestine of Nrf2 wild-type mice but not in small intestine of Nrf2 knockout mice comparing with vehicle treatment at both time points. The relative mRNA expression levels of each gene in treatment group over vehicle group (fold of change) were listed.

(*Continued on the following page*)

Table 3. Curcumin-suppressed Nrf2-dependent gene lists in liver and small intestine (Cont'd)

Gene description	Symbol	Genbank	Liver*		Small intestine †	
			3 h	12 h	3 h	12 h
Wingless-related mouse mammary tumor virus integration site 6	Wnt6	AV308073	0.35	0.34		
Wingless-related mouse mammary tumor virus integration site 7A	Wnt7a	BB129109	0.36	0.45		
Eph receptor A3	Epha3	M68515			0.39	0.26
Germ cell–specific gene 2	Gsg2	BE457839			0.49	0.34
Similar to PKCζ (LOC233024), mRNA	PrkcZ	BG143376			0.41	0.37
G protein-coupled receptors						
5-Hydroxytryptamine (serotonin) receptor 4	Htr4	Y09587	0.10	0.19		
5-Hydroxytryptamine (serotonin) receptor 2C	Htr2c	BQ174268	0.42	0.38		
G protein-coupled receptor 63	Gpr63	BB131092	0.23	0.19		
Olfactory receptor 17	Olfr17	NM_020598	0.42	0.41		
Platelet-derived growth factor receptor like	Pdgfrl	AK004179			0.35	0.47
Transcription factors						
Ankyrin repeat and SOCs box-containing protein 5	Asb5	NM_029569	0.10	0.44		
Ankyrin repeat domain 6	Ankrd6	BM199504	0.50	0.39		
Camello-like 3	Cml3	NM_053097	0.44	0.38		
Forkhead box F2	Foxf2	NM_010225	0.24	0.35		
Forkhead box M1	Foxm1	BB398835	0.32	0.34		
Hairy/enhancer-of-split related with YRPW motif 2	Hey2	NM_013904	0.18	0.15		
Homeobox B8	Hoxb8	X13721	0.45	0.05		
Homeobox, msh-like 2	Msx2	AV297190	0.29	0.10		
Jumonji domain containing 2C	Jmjd2c	BC020180	0.37	0.36		
Kruppel-like factor 3 (basic)	Klf3	BE687999	0.40	0.30		
Kruppel-like factor 5	Klf5	BC006646	0.30	0.40		
Paired like homeodomain factor 1	Prop1	NM_008936	0.48	0.30		
Snail homologue 2 (*Drosophila*)	Snai2	NM_011415	0.40	0.42		
D site albumin promoter-binding protein	Dbp	BC018323			0.40	0.06
Insulin promoter factor 1, homeodomain transcription factor	Lpf1	AK020261			0.37	0.26
Nuclear factor, erythroid derived 2, like 3	Nfe2l3	NM_010903			0.49	0.28
Period homologue 3 (*Drosophila*)	Per3	NM_011067			0.46	0.15
Retinoic acid receptor–related orphan receptor β	Rorb	BB751387			0.16	0.38
SRY box–containing gene 11	Sox11	BG072739			0.49	0.31
SRY box–containing gene 4	Sox4	AI428101			0.31	2.42
Zinc finger protein 354C	Zfp354c	BB024472			0.28	0.31
Others						
Chemokine (C-X-C motif) ligand 14	Cxcl14	AF252873	0.37	0.47		
Vitamin D receptor	Vdr	AV290079	0.12	0.50		

manner after curcumin treatment. The induction of nuclear factor-κB signaling pathway component gene *inhibitor of κB kinase γ* and suppression of Wnt signaling pathway-related *Wnt6* and *Wnt7a* genes were consistent with previous results (40–42). The suppression of phosphatidylinositol 3-kinase downstream target protein kinase C (PKC) ζ–related gene *Prkcz* suggested that curcumin may intervene in the phosphatidylinositol 3-kinase signaling and nuclear factor-κB p65 subunit nuclear translocation in small intestine (43). Because PKCμ could phosphorylate E-cadherin and increase prostate cancer cell aggregation and decrease cellular motility (38), the induction of both *PKCμ* gene and several cadherin genes by curcumin in small intestine may contribute to its colon cancer chemopreventive effect.

Because of the protective role of Nrf2-mediated gene expression in response to carcinogen or reactive oxygen intermediate challenge, it is essential and important to identify novel Nrf2/ARE pathway target genes related to cancer chemoprevention in addition to phase II detoxification and antioxidant genes (2, 16). By comparing the gene expression patterns elicited by promising cancer chemopreventive agent curcumin between Nrf2 wild-type and knockout mice, we identified many novel curcumin-regulated Nrf2-dependent genes with a variety of biological functions in mice liver and small intestine. The identification of these genes clearly expanded our scope of understanding the role of Nrf2 in cancer chemoprevention as well as potential new mechanisms of cancer chemoprevention. Interestingly, two previous microarray studies using chemopreventive agent sulforaphane (18) and 3H-1,2-dithiole-3-thione (17) to compare their gene expression profiles between wild-type and Nrf2-deficient mice also identified some of the similar functional categories of Nrf2-dependent genes. Although the chemopreventive agents used in

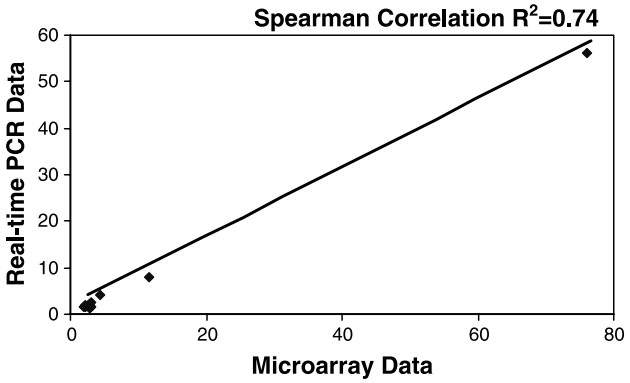

Figure 3. Correlation of microarray data and quantitative real-time PCR data. Fold of changes of gene expression measured by real-time PCR were plotted against the corresponding fold of changes in microarray data. The Spearman correlation was calculated as $R^2 = 0.74$, which indicated the data from the two methods were in good correlation.

previous studies and our current study are different, the similar induction pattern and the regulation of many identical Nrf2-dependent genes strongly suggested a relationship between the sets of genes being regulated and the cancer chemopreventive effects of these compounds as well as the predominant role of Nrf2 in the regulation of these genes. It also suggested that an elicited similar global gene expression change rather than the regulation of individual pathways could lead to the overall cancer protective effect by these different classes of chemopreventive compounds. Future *in vivo* or *in vitro* studies to explore the roles of Nrf2-dependent genes related to ubiquitination, drug metabolism, cell growth and adhesion, phosphorylation, and transcription as uncovered in our current study will greatly extend our knowledge on cancer chemoprevention.

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