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## An Approach to the Study of Gene Expression in Hepatocarcinogenesis Initiation<sup>1,2</sup>

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### Abstract

In carcinogenesis, determination of gene and protein expression profiles is important for prevention and treatment. Caffeic acid phenethyl ester (CAPE) in a single dose administered before carcinogenic initiation induced by diethylnitrosamine (DEN) prevents the appearance of preneoplastic lesions. On the basis of this approach, the main purpose of this work was to compare the gene expression profiles induced by DEN or a previously administered single dose of CAPE. Using a modified hepatocarcinogenesis-resistant hepatocyte model, male Fischer-344 rats were administered with one intraperitoneal dose of CAPE (20 mg/kg) 12 hours before DEN administration (200 mg/kg). Livers were removed and processed for microarray analysis and reverse transcription–polymerase chain reaction 12 hours after CAPE dosing and 24 hours after DEN administration with or without CAPE. CAPE alone did not alter the expression profile. DEN treatment modified the expression of 665 genes, and CAPE plus DEN induced changes in 1371 genes. DEN treatment increased the expression of genes associated with oxidative stress such as *glutathione reductase*, genes involved in cell cycle regulation including *p53*, and modified cytochrome P450. CAPE plus DEN diminished the expression of cytochrome involved in DEN bioactivation such as *CYP2B1* as well as the expression of regulators of oxidative stress such as *glutathione reductase*, *GST-κ* and *GST-θ*, and cell cycle regulators such as *p53*. Using CAPE as a tool, we uncovered new approaches for studying the altered expression of reactive genes and identifying proteins that will help to propose well-sustained and concrete hypothesis of DEN mechanism of hepatocarcinogenesis initiation.

*Translational Oncology (2010) 3, 142–148*

### Introduction

Exposure to chemical carcinogens is one of the most studied areas in carcinogenesis, and three major stages in carcinogenesis have been recognized: initiation, when mutations occur and initiated cells are generated; promotion, when clonal expansion of initiated cells takes place and forms preneoplastic lesions; and progression, when preneoplastic lesions become tumors through the gain of additional genetic and metabolic alterations [1].

Diethylnitrosamine (DEN), an environmental carcinogen, has been used in several experimental models [2]. Specifically, it has been used as an initiator in the modified resistant hepatocyte model [3,4] because initiation is necessary for carcinogenesis [5]. This effect has not yet been completely studied, with only a few elements of the pathway having been identified thus far. As early as 3 hours after DEN administration, lipid peroxidation can be detected [6], as well

as overexpression of glutathione-S-transferase Pi (*GST-p*) at 4 hours; this is considered a marker of initiation in chemical carcinogenesis and tumors [7]. In addition, the formation of DNA adducts have been detected as early as 3 hours after administration [8], and generalized necrosis clearly seen after 24 hours is a sign of subsequent damage [5,9].

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In the modified resistant hepatocyte model of hepatocarcinogenesis, it has been demonstrated that oxidative stress is necessary for initiation. When *N*-ethyl-*N*-nitrosourea is used as the initiator carcinogen instead of DEN, it generates the same adducts as DEN, but without an important oxidative stress. When *N*-ethyl-*N*-nitrosourea is used, preneoplastic lesions are not observed at 25 days, in contrast to the high number that occurs when oxidative stress and adducts are produced by DEN. This finding is also supported by the decreased induction of preneoplastic lesions by the antioxidant quercetin [6]. Oxidative stress generated during the initiation stage is mainly due to DEN metabolism by cytochrome P450 (CYP). DEN must be metabolized by several CYP family members to produce the active procarcinogen, and when the isoforms related to this bioactivation are inhibited by a chemoprotector such as caffeic acid phenethyl ester (CAPE), the lesions observed in the model are drastically reduced [5,10].

As previously shown in the modified resistance hepatocyte model, three stages are necessary to produce preneoplastic lesions at 25 days and liver cancer after 7 months of initiation [5,11]. As such, all components of this model (DEN, 2-acetyl aminofluorene administration and partial hepatectomy) are necessary, and it is implied that each has an individual pathological mechanism; however, the specific mechanisms of each step in carcinogenesis process remain unclear.

Gene expression profile (GEP) of the progression stage has shown important genetic changes related to the evolution of liver preneoplastic lesions toward neoplastic lesions and tumors [11]. However, GEP and possible expression modifications by anticancer agents have not been extensively studied.

The chemoprotectors quercetin, celecoxib, and CAPE are inhibitors of the initiation stage and prevent preneoplastic lesions; CAPE is one of the most efficient inhibitors. When administered 12 hours before DEN, this natural component of propolis diminished tissue damage produced 24 hours after DEN administration, as well as lipid peroxidation and modification of CYP isoforms 1A1/2 and 2B1/2 related to DEN bioactivation. All of these alterations are thought to be related to the reduced incidence of preneoplastic lesions at 25 days after DEN treatment in CAPE-pretreated animals [5,11].

Thus, the aim of this work was to analyze the initiation stage using microarrays to contrast the changes in GEP produced by DEN alone with those produced when CAPE was administered before DEN. We have hypothesized that the comparison of the carcinogenic treatment *versus* the chemoprotector plus carcinogen will show relevant changes in gene expression pertaining to the initiation of carcinogenesis.

Results indicated that there is a differential effect on GEP induced by CAPE with or without DEN. When CAPE was administered alone, it did not modify GEP. After DEN administration, 665 genes were modulated and CAPE plus DEN modified surprisingly 1371 genes. CAPE modulated several cell physiological aspects, such as DEN metabolism, regulation of oxidative stress, and others such as proliferation, DNA replication, and DNA repair. These results proposed new routes to study the principal events performed in the initiation of hepatocarcinogenesis process and in the chemoprotector action mechanism of CAPE.

## Materials and Methods

### Animals

Male Fischer-344 rats (180–200 g) were obtained from the Unit for Production of Experimental Laboratory Animals (UPEAL Cinvestav, Mexico City, Mexico). Animals had access to food (PMI Feeds, Inc,

Laboratories Diet, Richmond, IN) and water *ad libitum*, with each rat consuming approximately 12 to 15 g of food and 10 to 15 ml of water per day. Animals were maintained in a holding room under controlled conditions of 12-hour light/dark cycles, 50% relative humidity, and 21°C. Animal care followed institutional guidelines for the use of laboratory animals.

### Experimental Protocol

Animals were administered 200 mg/kg DEN (Sigma-Aldrich, St Louis, MO) as in the modified resistant hepatocyte model of Semple-Robert [3]. A group of rats was pretreated with a 20-mg/kg single dose of CAPE (kindly provided by Dr. Javier Hernández-Martínez, CIAD, Hermosillo, Mexico) 12 hours before DEN treatment [10]. Animals were killed 12 hours after CAPE alone and 24 hours after DEN administration with and without CAPE. Animals were killed by exsanguination. Livers were excised, washed in physiological saline solution, frozen in 2-methyl butane with liquid nitrogen, or immersed in RNAlater (Sigma) and stored at –80°C. Frozen livers sections were used to complementary DNA (cDNA) microarray and reverse transcription–polymerase chain reaction (RT-PCR) assays ( $n = 4$  in each group).

### cDNA Microarray Slides

Microarrays were assayed at the Biochips Platform of Genopole, University of Toulouse, INSA, UPS, INP & INRA (Toulouse, France). cDNA hybridization was performed on slides with 28,800 spots using 27,004 oligonucleotides of 22,012 rat genes, including 5000 genes with known function in the rat. Data were analyzed with the Web service Biplot, Bioclust, and Venn diagrams from Transcriptome-Biochips Platform of Genopole Toulouse Midi-Pyrenees (<http://biopuce.insa-toulouse.fr>). Altered genes were selected using a ratio of up-regulation threshold greater than 1.5, down-regulation threshold less than 0.6, and Student's *t* test with  $P < .05$  considered statistically significant. The generated data set has been submitted to the National Center for Biotechnology Information Gene Expression Omnibus (GEO, <http://www.ncbi.nlm.nih.gov/geo/index.cgi>) database (GSE12030).

### Relative Quantitative RT-PCR

Total RNA extraction was performed using Tripure Isolation Reagent (Roche Diagnostics, Indianapolis, IN). DNase treatment was performed using RNase-free DNase I (Roche Diagnostics), and cDNA synthesis was performed using SuperScript Reverse Transcriptase (Invitrogen, Carlsbad, CA). Real-time PCR was performed in a LightCycler Carousel-Based System Instrument 2.0 (Roche Diagnostics) using LightCycler FastStart DNA MasterPLUS SYBR Green I (Roche Diagnostics), and primers were synthesized by Invitrogen (Table 1). RT-PCR was performed in quadruplicate, and additional reactions were performed without reverse transcriptase to verify the absence of DNA contamination. Gene expression quantification was performed using a derivation of the  $2^{-\Delta\Delta C_T}$  method,  $2^{-\Delta C_T}$  [12]. Student's *t* test was used as statistical analysis.

## Results

### Differential GEPs in Liver Cancer Initiation

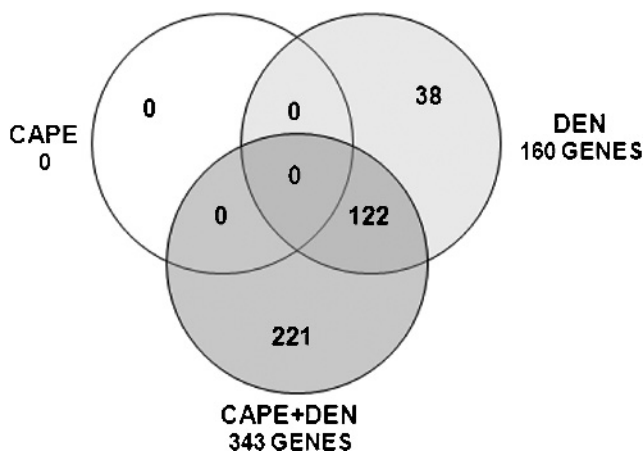
Considering that omission of the carcinogen initiator is sufficient to avoid preneoplastic lesions and cancer induction [5], molecular

**Table 1.** Primers Used in RT-PCR.

Symbol	Gene ID	Forward	Reverse	Size (bp)
<i>CYP1A2</i>	NM_012541	AGGGACACCTCACTGAATGG	CCGAAGAGCATCACCTTCTC	182
<i>CYP2A2</i>	NM_012693	ATCCAGATGTGGAAGCCAAG	CCACGGAAAGTTGTGTCTT	187
<i>CYP2B1</i>	NM_001134844	GGAAGCTCTGGTTGCTTGAC	CAAAGAAGTGCAGACCGACA	206
<i>CYP2E1</i>	NM_031543	CCTACATGGATGCTGTGGTG	CTGGAAACTCATGGCTGTCA	171
<i>GST-θ</i>	NM_012796	ATGGCATTCCCTTTCAGTTG	GTGGTCTGCCACCTGGTACT	179
<i>GST-κ</i>	NM_181371	CACGGAGTCCCAGAACAATTT	CGGTCCAGACCCAAATAGCAT	210
<i>p53</i>	NM_030989	TCTCCAGCAAAAGAAAAA	CTTCGGGTAGCTGGAGTGAG	168
<i>p38</i>	NM_031020	AGCGATACCAGAACCTGTCC	GAACGTGGTCACTCGGTAAGC	318
<i>YY1</i>	NM_173290	GACGACGACTACATCGAGCA	TTGATCTGCACCTGCTTCTG	209
<i>DNApolδ</i>	NM_021662	GACGACGACTACATCGAGCA	AGCGGGAGGAGAAGAGTAGG	217
<i>GLUTATHION REDUCTASE</i>	NM_053906	CCATGTGGTTACTGCACCTCC	GTTCCITTCCTTCTCTGAGC	171

These genes were selected from microarrays analysis and validated by RT-PCR.

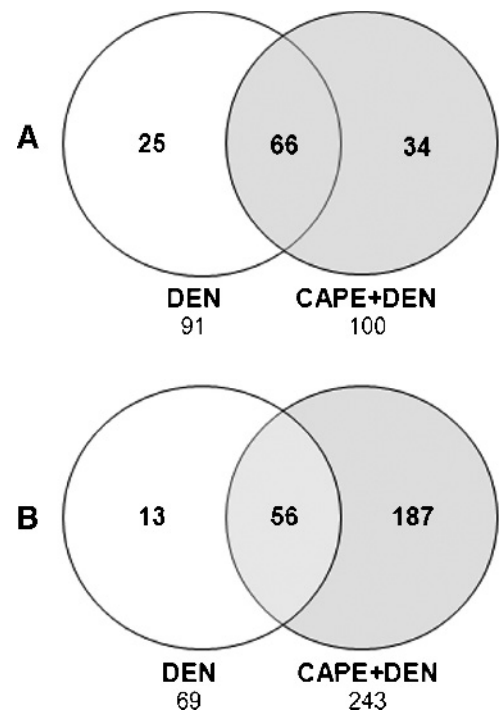
alterations at the initiation stage are likely necessary for cancer to occur. To characterize the GEP modifications that occur at the initiation stage, microarray analyses were performed to determine genes differentially expressed on DEN, CAPE, or CAPE plus DEN administration. At 24 hours after DEN administration, 665 genes were transcriptionally modified; of these, 160 had known function in the rat, with 91 upregulated and 69 downregulated. The effect of CAPE was analyzed at 12 hours after administration; this elapsed period before DEN administration was used to determine GEP changes produced by the chemoprotector alone. As such, CAPE administration did not induce modifications in the GEP. When CAPE was administered 12 hours before DEN and its effect evaluated 24 hours after DEN treatment, the expression of 1371 genes was changed; 343 of these have known function in the rat. Of these genes, 100 were upregulated and 243 were downregulated. Of the 160 altered genes with known function in the rat, 38 were restored to normal levels, 122 remained altered with DEN treatment alone, and the expression of 221 was newly modified in the CAPE plus DEN group (Figure 1). It is tempting to speculate that genes whose expression was initially modified by DEN were restored by CAPE treatment thus describing a chemoprotective mechanism for CAPE.



**Figure 1.** Venn diagram of transcriptionally altered genes. Data were analyzed using the Venn diagram program from <http://biopuce.insa-toulouse.fr>. Genes were identified as upregulated (threshold > 1.5) or downregulated (threshold < 0.6) with  $P < .05$  considered statistically significant with Student's  $t$  test ( $n = 4$  in each group).

### Upregulated Genes

A general analysis of GEP was the starting point for this study. In the DEN-treated group, 91 genes were upregulated; of these, 66 remained modified after CAPE treatment (Table W1), whereas the expression of 25 was restored to untreated levels (Table W2). The 91 genes modified by DEN included several *ribosomal proteins*, *CYP*, *heat shock proteins*, and genes such as *GAPDH* and *GST-κ*, suggesting an important alteration of protein synthesis, metabolism, and oxidative stress, which are all involved the initiation stage (Table W1). In the CAPE plus DEN group, 100 genes were upregulated; of these, 66 were upregulated after DEN treatment (Table W1) and 34 were only upregulated after CAPE plus DEN administration (Table W3). These 34 genes encode ribosomal proteins as well as *heat*



**Figure 2.** Comparison of Venn diagrams for genes upregulated or downregulated after DEN or DEN plus CAPE treatment. (A) Upregulated genes, threshold greater than 1.5 with  $P < .05$  by Student's  $t$  test. (B) Downregulated genes, threshold less than 0.6 with  $P < .05$  by Student's  $t$  test. Data were analyzed using the Venn diagram program from <http://biopuce.insa-toulouse.fr> ( $n = 4$  in each group).

**Table 2.** Genes Validated by RT-PCR for Microarray Results.

Gen	Function
<i>CYP2B1</i> *	DEN metabolism
<i>CYP1A2</i> *	
<i>CYP2A2</i> *	
<i>CYP2E1</i>	
<i>GST-κ</i> †	Oxidative stress regulation
<i>GST-θ</i> *	
<i>GLUTATHIÓN REDUCTASE</i> *	
<i>YY1</i> *	Replication DNA repair Cell signaling process.
<i>DNA polδ</i> *	
<i>p53</i> *	
<i>p38</i> *	

\* Genes downregulated by CAPE plus DEN as determined by microarray.

† Genes upregulated by DEN as determined by microarray.

*shock proteins* and *CYP*, among others. In line with chemoprotective effects of CAPE, there were differences between in GEPs between both groups (Figure 2A).

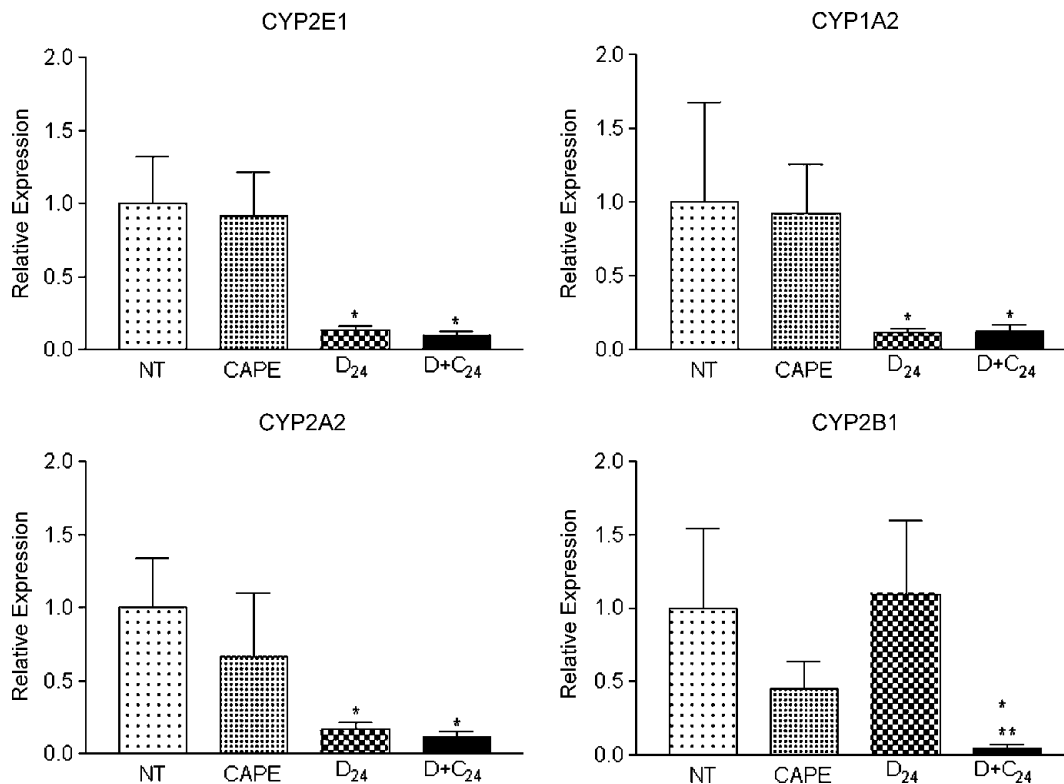
**Downregulated Genes**

After DEN treatment, 69 genes were downregulated, and 56 genes remained downregulated after CAPE treatment (Table W4). Of these, the expression of 13 was restored to control levels by CAPE administration before DEN (Table W5). Within the set of downregulated genes, there were several *CYP isoforms* as well as *microglobulins*, *macro-globulins*, *VEGFR1*, and *APC*. In general, gene down-regulation was a hallmark of CAPE pretreatment because CAPE plus DEN treatment downregulated 243 genes, of which 56 were common to the DEN alone condition and 187 genes were downregulated independently

of DEN administration (Table W6), including *ribosomal proteins*, *CYP*, *globulins*, *p53*, *p38*, *transcription factor YY1*, *GST-θ*, and others. It should be noted that some CYP family members were down-regulated in the CAPE plus DEN group, including bioactivators of DEN such as *CYP2E1*, *CYP2B1*, *CYP1A2*; this suggests that CAPE treatment might affect DEN bioactivation. In addition, CAPE might act by modifying the cell cycle, oxidative stress, and other pathways involved in cell regulation (Figure 2B).

**PCR Validation**

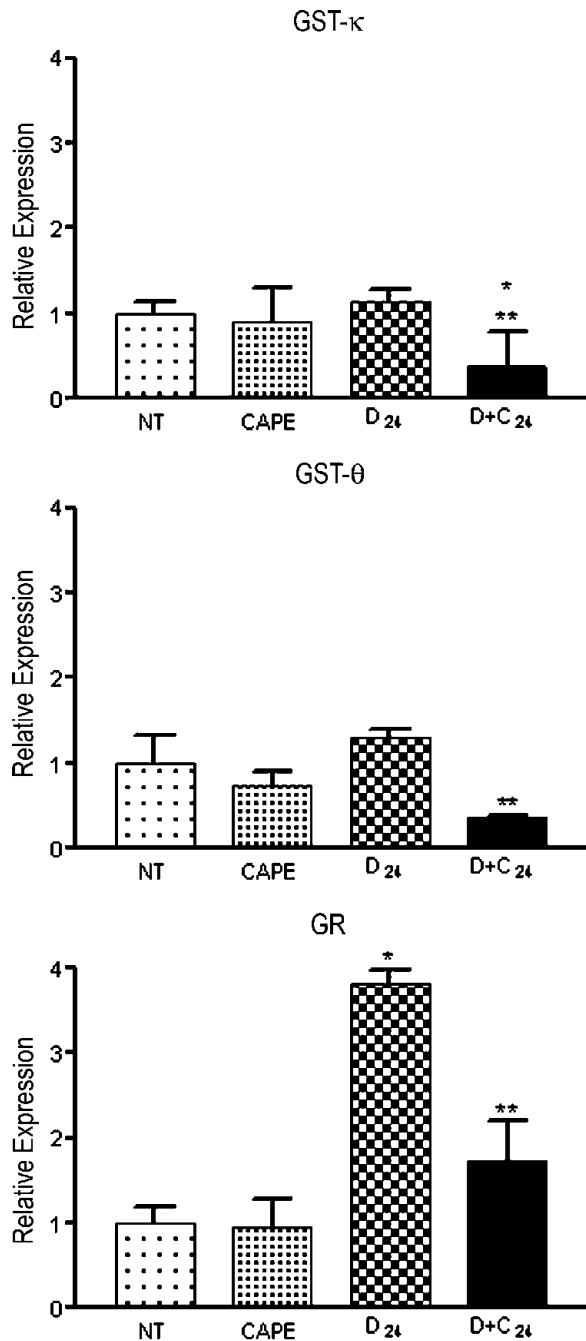
The aim of using microarrays was to explore the GEP to generate hypothesis about events involved in tumor initiation. Microarrays are completely reliable because the genes are not individually evaluated. Thus, microarray results must be validated using a quantitative method. For RT-PCR validation, we selected 11 genes (Table 2): four related to DEN metabolism (*CYP2E1*, *2B1*, *1A2*, and *2A2*), three related to oxidative stress regulation (*GST-κ*, *GST-θ*, and *glutathione reductase*), and four related to cell regulation (*transcription factor YY1*, *DNA polymerase δ [DNA polδ]*, *p53*, and *p38*). We found that DEN administration decreased *CYP2E1*, *1A2*, and *2A2* expression, but CAPE administration did not ameliorate this effect. Conversely, *CYP2B1* expression was not altered by DEN, but at 24 hours after DEN treatment, CAPE administration significantly decreased the expression of this isoform (Figure 3). The expression of *GST-κ*, *GST-θ*, and *glutathione reductase* genes increased with DEN administration and decreased in the CAPE plus DEN group (Figure 4). DEN treatment increased *YY1*, *p53*, and *DNA polδ* expression; this effect decreased with CAPE pretreatment. DEN reduced the expression of



**Figure 3.** RT-PCR validation of *CYP* expression levels. Samples were analyzed by RT-PCR using primers described in Table 1. CAPE indicates rats killed 12 hours after CAPE dosing; D + C<sub>24</sub>, killed 24 hours after DEN dosing with CAPE pretreatment 12 hours before DEN (n = 4 in each group); D<sub>24</sub>, killed 24 hours after DEN dosing; NT, untreated. \*P < .05 compared with NT, \*\*P < .05 compared with D<sub>24</sub> by Student's t test.



*p38* and CAPE had no effect (Figure 5). This suggests that CAPE modulates the alterations produced by DEN at different levels. Furthermore, these results confirm the importance of microarray validation, because on RT-PCR analysis, the expression of some genes was not in complete agreement with microarray data. We also observed expression changes that were not observed when using the microarray, such as increased expression of *YY1*, *p53*, and *DNA polδ* genes.



**Figure 4.** RT-PCR validation of *GST-κ*, *GST-θ*, and *glutathione reductase (GR)* expression levels. Samples were analyzed by RT-PCR using primers from Table 1. CAPE indicates rats killed 12 hours after CAPE dosing; D + C<sub>24</sub>, killed 24 hours after DEN dosing with CAPE pretreatment 12 hours before DEN (*n* = 4 in each group); D<sub>24</sub>, killed 24 hours after DEN dosing; NT, untreated. \**P* < .05 compared with NT, \*\**P* < .05 compared with D<sub>24</sub> by Student's *t* test.

## Discussion

Chemoprevention is helpful for preventing or even curing cancer in any of the three stages of carcinogenesis: initiation, promotion, or progression. To this end, CAPE has been studied for the past 20 years, and it has shown chemoprotective properties in several types of cancer. In different experimental models, the mechanism of action differs depending on the situation tested [4,5,10,12–19]. In the modified resistant hepatocyte model of liver cancer, the chemoprotective mechanism of action of CAPE in the initiation stage is not completely understood, but it seems to be related to the inhibition of DEN bioactivation [5].

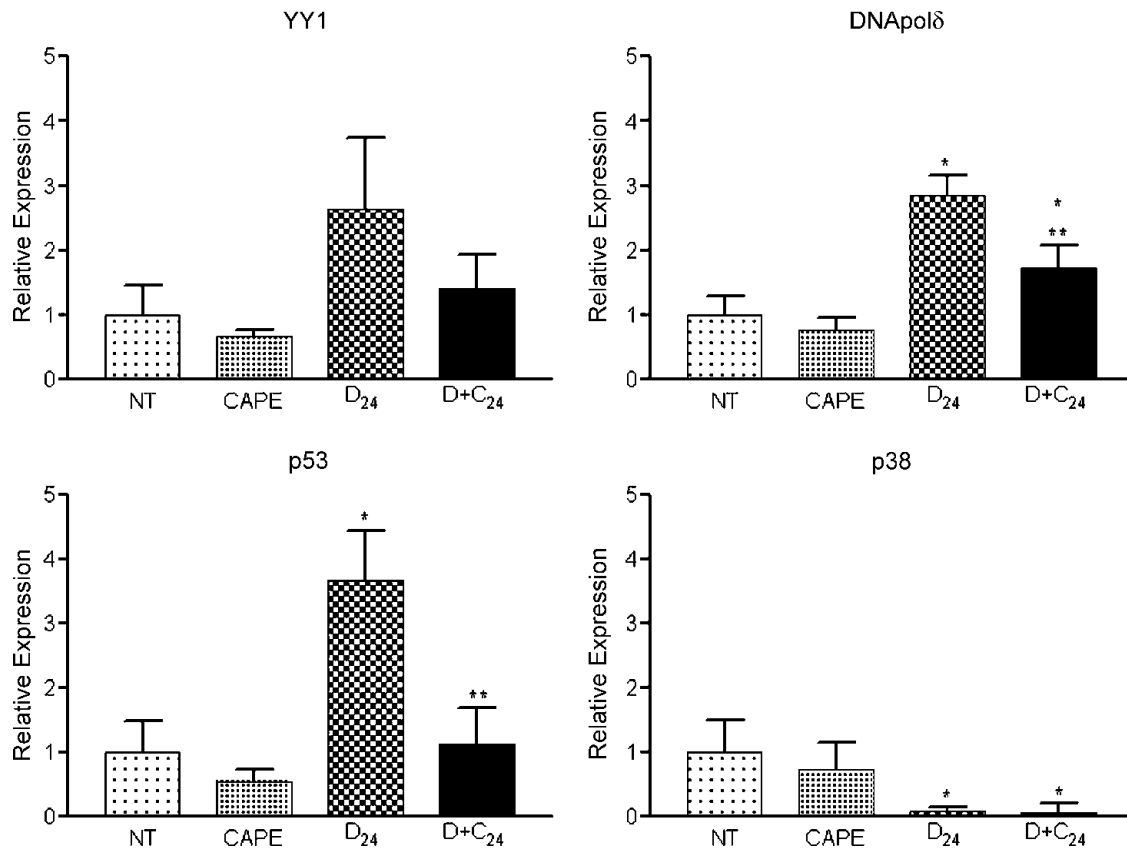
The initiation stage is a necessary step in the modified resistant hepatocyte model. Initiation is caused by DEN administration. The metabolites produced during bioactivation produce mutations in DNA, generate oxidative stress, and, finally, initiate cells toward transformation. Furthermore, oxidative stress and DNA alkylation have both been suggested to be necessary for initiation [6]. During the first 24 hours after DEN administration, the expression of the tumor marker *GST-p* is observed, and lipid peroxidation, adduct formation, CYP modifications, and tissue damage are also detected [5–8]. These alterations thus represent a phenotype characteristic of initiated hepatocytes. However, it is important to identify the immediate early changes in hepatocarcinogenesis to uncover specific molecular targets for detection and chemoprevention.

Microarray analysis is a powerful technique for genetic and genomic investigation, making it possible to simultaneously analyze gene expression of thousand of genes [20]. This is important, considering that gene expression variations are associated with common diseases and do not lead directly to disease, but instead generate an intermediary molecular phenotype. Therefore, it is important to identify the molecular phenotypes associated with changes in disease state. In this manner, molecular networks that involve changes in disease can be elucidated [21].

We used microarray slides with 28,800 spots, allowing for a large and complex data set to be analyzed. It was necessary to further select a small group of genes to validate. We started with genes with well-known function in rats. From the analysis of 28,800 spots, we identified genes expressed differentially in each treatment; 160 were modified by DEN, 343 by DEN plus CAPE, and none by CAPE alone before DEN administration.

To validate microarrays by RT-PCR, we selected three groups of genes related to specific functions in the rat liver that could be involved in the initiation process. Genes that 1) might be involved in the initiation of carcinogenesis, 2) are directly involved in the initial oxidative stimulus that modifies the phenotype of hepatocytes, and 3) activate possible proliferation signals through DNA alkylation were selected. The first genes selected were those related to DEN bioactivation because alteration of DEN metabolism should result in altered initiation. Gene expressions of *1A2*, *2E1*, and *2A2* isoforms were diminished by DEN treatment and were not modified by CAPE pretreatment. The expression of *2B1* was not affected by DEN but was significantly reduced by pretreatment with CAPE alone and by administration of both CAPE and DEN. Because CYP2B1 is an important bioactivator of DEN, we propose that DEN bioactivation by CYP2B1 is diminished by CAPE, as previously shown enzymatically [5].

The second group of genes selected were those involved in oxidative stress regulation including *GST-κ*, a mitochondrial GST isoform, *GST-θ*, a cytosolic isoform, and *glutathione reductase* [22]. These genes were upregulated by DEN treatment in agreement with the previous report of altered redox state [6]. The messenger RNA up-regulation of



**Figure 5.** RT-PCR validation of *YY1*, *DNA polδ*, *p53*, and *p38* gene expression levels. Samples were analyzed by RT-PCR using primers from Table 1. *CAPE* indicates rats killed 12 hours after *CAPE* dosing; *D + C<sub>24</sub>*, killed 24 hours after *DEN* dosing with *CAPE* pretreatment 12 hours before *DEN* ( $n = 4$  in each group); *D<sub>24</sub>*, killed 24 hours after *DEN* dosing; *NT*, untreated. \* $P < .05$  compared with *NT*, \*\* $P < .05$  compared with *D<sub>24</sub>* by Student's *t* test.

these genes was not present with *CAPE* pretreatment alone or in the *CAPE* plus *DEN* group. This suggests that pretreatment-derived counteraction of oxidative stress is likely related to the decreased bioactivation of *DEN*; this effect is likely derived from the reduced generation of oxidative stress by *CYP* together with the direct antioxidant activity of *CAPE* [10]. Both mechanisms deserve further analysis because there is a need to identify new candidates as possible initiation markers during the early stages of carcinogenesis, such as *GST-p* [7].

The third group of selected genes was related to cell regulation processes such as proliferation and DNA repair. The transcription factor *YY1* participates in many aspects of cell regulation including activation, repression, and modification of growth, development, and differentiation GEPs [23]. *DNA polδ* participates in DNA replication and DNA repair processes including base pair excision, nucleotide excision, and mismatch repair [24]. *p53* protects the genomic integrity by participating in cell cycle arrest, DNA repair, and apoptosis, and it has been found to be overexpressed in hepatocellular carcinoma [25]. After validating the GEPs, we observed that *p38* expression, a mitogen-activated protein kinase, was diminished with *DEN* treatment and was not restored by *CAPE* [26]. The expression of *p53*, transcription factor *YY1*, and *DNA polδ* was increased by *DEN* treatment, suggesting altered DNA repair and replication. Up-regulation of these genes was avoided by *CAPE* pretreatment. After *DEN* bioactivation, DNA damage is followed by DNA repair and replication; however, if *DEN* is not completely metabolized, the DNA damage is decreased. These effects are likely mediated directly by *CAPE*, with

subsequently reduced expression of *YY1* and *p53*; the transcription factor *YY1* regulates the transcription of *p53* [23]. Further investigation is required to determine the participation of these genes and their protein products during carcinogenesis initiation.

There are many more genes that could be selected for further analysis from our microarray; thus, our analysis only represents an initial effort to identify the genes participating in the initiation stage of this model of chemical hepatocarcinogenesis.

Other groups of genes relevant for future validation and investigation include those encoding heat shock proteins, which are molecular chaperones induced by several pathological stimuli. Alterations in processing proteins have been related to the carcinogenesis process; these proteins have been found to be overexpressed in gastric cancer, and they serve as possible markers of tumorigenesis, malignant phenotype, tumor immunity, resistance to apoptosis, and poor prognosis [27]. Several *CYP* family members seemed to be downregulated after *DEN* administration; these include *3A1* and *2C11*, which have been previously reported to be downregulated most likely as the result of tissue damage produced by *DEN* [28]. *CYP* down-regulation is expected in the response to inflammation and infection. As consequence of generalized necrosis observed at 24 hours after *DEN*, the inflammation may have contributed to *CYP* down-regulation, as previously reported for *CYP* isoforms such as *2C11*, *3A2*, and *2E1* [29,30]. In addition, several microglobulins and macroglobulins were downregulated, specifically  $\alpha$ -2 $\mu$ -globulins that might be involved in increased *DEN*-mediated proliferation as previously reported [31].

When CAPE was administered alone, it did not modify gene expression; but when DEN was administered after CAPE, a dramatic response was obtained when compared with that produced by the carcinogen alone. This induction of a protective phenotype by CAPE is similar to a previously observed differentiation of effects between normal and altered cells, where CAPE produced radiosensitization in lung cancer [12], CT26 colorectal adenocarcinoma cells [13], and preferential cytotoxicity in several cancer cells but not in normal ones [15]. This intriguing effect of CAPE should be further investigated.

It would seem that tumor initiation by DEN induces changes in GEP. These changes probably influence protein modifications. CAPE given before DEN interferes with DEN-mediated expression changes in genes involved in DEN bioactivation, oxidative stress regulation, DNA repair and replication, among others. The effect of CAPE on this process can be used as a tool to suggest new mechanisms for DEN initiation process in the modified resistant hepatocyte model as well as to uncover possible molecular targets for liver cancer chemoprotection.

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**Table W1.** Upregulated Genes by DEN Even with CAPE Pretreatment.

Gene Name	Ratio
1 Zinc finger protein 5 (fragment). [Source: SPTREMBL; Acc: P97661]	2.116
2 Neuronal acetylcholine receptor protein, alpha-7 chain precursor. [Source: SWISSPROT; Acc: Q05941]	1.866
3 Rab6-interacting protein 2; rim-binding protein; ELKS. [Source: RefSeq; Acc: NM_170788]	1.772
4 40S ribosomal protein S5. [Source: SWISSPROT; Acc: P24050]	1.648
5 Zinc finger protein 10 (fragment). [Source: SPTREMBL; Acc: P97649]	1.994
6 Ring finger protein OIP1. [Source: RefSeq; Acc: NM_134467]	2.615
7 40S ribosomal protein SA (P40) (34/67 kDa laminin receptor). [Source: SWISSPROT; Acc: P38983]	1.778
8 DNA-directed RNA polymerase I 135 kDa polypeptide (EC 2.7.7.6) (RNA polymerase I subunit 2) (RPA135) (RNA polymerase I 127 kDa subunit). [Source: SWISSPROT; Acc: O54888]	1.652
9 Cytochrome P450 27, mitochondrial precursor (EC 1.14.--) (cytochrome P-450C27/25) (sterol 26-hydroxylase) (sterol 27-hydroxylase) (vitamin D(3) 25-hydroxylase) (5-beta-cholestane-3-alpha,7-alpha,12-alpha-triol 27-hydroxylase). [Source: SWISSPROT; Acc: P1717]	1.584
10 60S ribosomal protein L13A. [Source: SWISSPROT; Acc: P35427]	1.862
11 Histamine H3 receptor (HH3R). [Source: SWISSPROT; Acc: Q9QYN8]	2.138
12 6-Phosphofructo-2-kinase/fructose-2,6-biphosphatase 3 (6PF-2-K/FRU-2,6-P2ASE brain-type isozyme) (RB2K) [includes: 6-phosphofructo-2-kinase (EC 2.7.1.105); fructose-2,6-bisphosphatase (EC 3.1.3.46)]. [Source: SWISSPROT; Acc: O35552]	1.784
13 Chymotrypsinogen B precursor (EC 3.4.21.1). [Source: SWISSPROT; Acc: P07338]	2.284
14 UDP-glucose 4-epimerase (EC 5.1.3.2) (galactowaldenase) (UDP-galactose 4-epimerase). [Source: SWISSPROT; Acc: P18645]	1.659
15 Zinc finger protein OZF (POZF-1). [Source: SWISSPROT; Acc: Q62981]	2.059
16 Epoxide hydrolase 1 (EC 3.3.2.3) (microsomal epoxide hydrolase) (epoxide hydratase). [Source: SWISSPROT; Acc: P07687]	2.329
17 60S ribosomal protein L35A. [Source: SWISSPROT; Acc: P04646]	1.647
18 40S ribosomal protein S17. [Source: SWISSPROT; Acc: P04644]	1.813
19 60S acidic ribosomal protein P2. [Source: SWISSPROT; Acc: P02401]	2.007
20 Eosinophil granule major basic protein precursor (MBP). [Source: SWISSPROT; Acc: Q63189]	1.657
21 Zinc finger protein 5 (fragment). [Source: SPTREMBL; Acc: P97644]	2.412
22 Dynein-like protein 10 (fragment). [Source: SPTREMBL; Acc: Q63161]	1.999
23 60S ribosomal protein L9. [Source: SWISSPROT; Acc: P17077]	1.711
24 HMGIC fusion partner-like protein 4. [Source: RefSeq; Acc: NM_181387]	1.622
25 Brain-enriched SH3-domain protein (BESH3). [Source: RefSeq; Acc: NM_139334]	1.678
26 ATP-binding cassette, subfamily C (CFTR/MRP), member 3. [Source: RefSeq; Acc: NM_080581]	2.251
27 ELAC homolog 2. [Source: RefSeq; Acc: NM_172326]	1.512
28 ER transmembrane protein DRI 42. [Source: RefSeq; Acc: NM_138905]	2.282
29 Cathepsin L precursor (EC 3.4.22.15) (major excreted protein) (MEP) (cyclic protein-2) (CP-2). [Source: SWISSPROT; Acc: P07154]	2.346
30 60S ribosomal protein L18. [Source: SWISSPROT; Acc: P12001]	1.861
31 Glyceraldehyde 3-phosphate dehydrogenase (EC 1.2.1.12) (GAPDH) (38 kDa BFA-dependent aDP-ribosylation substrate) (BARS-38). [Source: SWISSPROT; Acc: P04797]	1.554
32 High mobility group protein 2 (HMG-2). [Source: SWISSPROT; Acc: P52925]	2.395
33 40S ribosomal protein S27. [Source: SWISSPROT; Acc: P24051]	1.843
34 A-Kinase anchor protein 8 (A-kinase anchor protein 95 kDa) (AKAP 95). [Source: SWISSPROT; Acc: Q63014]	1.605
35 Parathyroid hormone receptor precursor (PTH2 receptor). [Source: SWISSPROT; Acc: P70555]	2.140
36 ARF GTPase-activating protein GIT1 (G protein-coupled receptor kinase-interactor 1). [Source: SWISSPROT; Acc: Q9Z272]	1.710
37 Gastrin precursor. [Source: SWISSPROT; Acc: P04563]	1.743
38 Taste receptor type 2 member 23 (T2R23) (taste receptor type 2 member 2) (T2R2). [Source: SWISSPROT; Acc: Q9JKF0]	2.103
39 Cell division cycle 5-like; CDC5 (cell division cycle 5, <i>Schizosaccharomyces pombe</i> , homolog)-like. [Source: RefSeq; Acc: NM_053527]	1.538
40 Solute carrier family 21 member 2 (prostaglandin transporter) (PGT) (matrin F/G). [Source: SWISSPROT; Acc: Q00910]	1.553
41 Peripheral plasma membrane protein CASK (EC 2.7.1.--) (calcium/calmodulin-dependent serine protein kinase). [Source: SWISSPROT; Acc: Q62915]	2.207
42 Zinc finger protein 16 (fragment). [Source: SPTREMBL; Acc: P97672]	1.923
43 40S ribosomal protein S21. [Source: SWISSPROT; Acc: P05765]	1.620
44 L-Lactate dehydrogenase A chain (EC 1.1.1.27) (LDH-A) (LDH muscle subunit) (LDH-M). [Source: SWISSPROT; Acc: P04642]	1.524
45 Peroxisome proliferator-activated receptor gamma coactivator 1β-2A. [Source: RefSeq; Acc: NM_176075]	2.008
46 Stromelysin-1 precursor (EC 3.4.24.17) (matrix metalloproteinase-3) (MMP-3) (TRANSIN-1) (SL-1) (PTR1 protein). [Source: SWISSPROT; Acc: P03957]	1.591
47 Ornithine decarboxylase (EC 4.1.1.17) (ODC). [Source: SWISSPROT; Acc: P09057]	1.781
48 Prolactin regulatory element-binding protein.	1.726
49 Linker for activation of T cells (36 kDa phospho-tyrosine adaptor protein) (PP36) (P36-38). [Source: SWISSPROT; Acc: O70601]	1.965
50 Protein kinase LYK5. [Source: RefSeq; Acc: NM_182820]	1.639
51 Gonadotropin-inducible ovarian transcription factor 2. [Source: RefSeq; Acc: NM_133390]	1.868
52 Tripeptidyl-peptidase II (EC 3.4.14.10) (TPP-II) (tripeptidyl aminopeptidase) (cholecystokinin-inactivating peptidase). [Source: SWISSPROT; Acc: Q64560]	1.906
53 Osteomodulin precursor (osteoaderin) (OSAD) (keratan sulfate proteoglycan osteomodulin) (KSPG osteomodulin). [Source: SWISSPROT; Acc: Q9Z1S7]	1.801
54 Tropomyosin isoform 6. [Source: RefSeq; Acc: NM_173111]	1.796
55 Heat shock protein HSP 90-beta (HSP 84). [Source: SWISSPROT; Acc: P34058]	1.992
56 Chemokine (C-C) receptor 4. [Source: RefSeq; Acc: NM_133532]	1.502
57 Class I MHC heavy chain RT1.A(N) antigen precursor. [Source: SPTREMBL; Acc: P79588]	1.674
58 Max interacting protein 1 (MXI1 protein). [Source: SWISSPROT; Acc: O09015]	1.718
59 BMP/retinoic acid-inducible neural-specific protein 3. [Source: RefSeq; Acc: NM_173121]	1.641
60 Prothymosin α. [Source: SWISSPROT; Acc: P06302]	1.840
61 Cadherin-4 (retinal-cadherin) (R-cadherin) (R-CAD) (fragment). [Source: SWISSPROT; Acc: Q63149]	2.415
62 Bone morphogenetic protein 1 (fragment). [Source: SPTREMBL; Acc: Q91WZ0]	1.653
63 Vitamin D-dependent calcium-binding protein, intestinal (CABP) (calbindin D9K) (cholecalciferin). [Source: SWISSPROT; Acc: P02634]	1.573
64 Hormone-sensitive lipase (EC 3.1.1.--) (HSL). [Source: SWISSPROT; Acc: P15304]	1.698
65 Metastasis-associated protein MTA1. [Source: SWISSPROT; Acc: Q62599]	1.545
66 Obscurin (fragment). [Source: SPTREMBL; Acc: Q80ZF5]	1.737

This list was selected with Biplot (Web site of Biopuce) upregulated threshold = 1.5,  $P < .05$ .

**Table W2.** Upregulated Genes by DEN Restored to Profile Level of Non Treated Rats with CAPE Pretreatment.

Gene Name	Ratio
1 Zinc finger protein 37 (ZFP-37). [Source: SWISSPROT; Acc: O88553]	1.760
2 Zinc finger protein 1 (fragment). [Source: SPTREMBL; Acc: P97657]	1.926
3 Serologically defined breast cancer antigen NY-BR-16-like protein (fragment). [Source: SPTREMBL; Acc: Q99MD2]	1.636
4 Amiloride-sensitive sodium channel gamma-subunit (epithelial Na <sup>+</sup> channel gamma subunit) (gamma ENAC) (non-voltage-gated sodium channel 1 gamma subunit) (SCNEG) (gamma NACH). [Source: SWISSPROT; Acc: P37091]	1.538
5 Cytochrome b-245 light chain (P22 phagocyte B-cytochrome) (neutrophil cytochrome b, 22 kDA polypeptide) (P22-PHOX) (cytochrome B(558) alpha chain) (cytochrome b558 alpha-subunit) (superoxide-generating NADPH oxidase light chain subunit). [Source: SWISSPROT; Acc: Q61462]	1.592
6 Copper transport protein ATOX1 (metal transport protein ATX1). [Source: SWISSPROT; Acc: Q9WUC4]	1.509
7 Kinesin-related protein 3A (fragment). [Source: SPTREMBL; Acc: O54720]	1.568
8 Cathepsin R. [Source: RefSeq; Acc: NM_175581]	1.595
9 CCAAT-binding transcription factor subunit B (CBF-B) (NF-Y protein chain A) (NF-YA) (CAAT-Box DNA binding protein subunit A). [Source: SWISSPROT; Acc: P18576]	1.589
10 Splicing factor 1 homolog (fragment).	1.524
11 60S ribosomal protein L26. [Source: SWISSPROT; Acc: P12749]	1.533
12 Transcription factor MTSG1. [Source: RefSeq; Acc: NM_178093]	1.567
13 Potassium voltage-gated channel subfamily G member (potassium channel K <sub>v</sub> 6.3) (K <sub>v</sub> 10.1). [Source: SWISSPROT; Acc: Q8R523]	1.606
14 Disrupted in schizophrenia 1. [Source: RefSeq; Acc: NM_175596]	1.515
15 Pius protein. [Source: RefSeq; Acc: NM_021660]	1.562
16 Cytochrome <i>c</i> oxidase polypeptide VIIA-liver/heart, mitochondrial precursor (EC 1.9.3.1) (cytochrome <i>c</i> oxidase subunit VIIA-L). [Source: SWISSPROT; Acc: P35171]	1.534
17 Neuronal tropomodulin (N-TMOD) (tropomodulin 2). [Source: SWISSPROT; Acc: P70566]	1.713
18 3,2- <i>trans</i> -enoyl-CoA isomerase, mitochondrial precursor (EC 5.3.3.8) (dodecenoyl-CoA delta-isomerase). [Source: SWISSPROT; Acc: P23965]	1.605
19 60S ribosomal protein L12. [Source: SWISSPROT; Acc: P23358]	1.502
20 Prokineticin 2 precursor (PK2). [Source: SWISSPROT; Acc: Q8R413]	1.511
21 Troponin T1, skeletal, slow. [Source: RefSeq; Acc: NM_134388]	1.516
23 Chloride channel protein, skeletal muscle (chloride channel protein 1) (CLC-1). [Source: SWISSPROT; Acc: P35524]	1.697
24 Neuroendocrine convertase 2 precursor (EC 3.4.21.94) (NEC 2) (PC2) (prohormone convertase 2) (proprotein convertase 2) (KEX2-like endoprotease 2). [Source: SWISSPROT; Acc: P28841]	1.621
25 Glutathione <i>S</i> -transferase, mitochondrial (EC 2.5.1.18) (GST 13-13) (glutathione <i>S</i> -transferase subunit 13) (GST class-kappa). [Source: SWISSPROT; Acc: P24473]	1.530

This list was selected with Biplot (Web site of Biopuce) upregulated threshold = 1.5,  $P < .05$  with Student's *t* test.

**Table W3.** Upregulated Genes Only When There Is CAPE Pretreatment before DEN.

Gene Name	Ratio
1 Prominin; fudenine. [Source: RefSeq; Acc: NM_021751]	1.537
2 $\beta$ Platelet-derived growth factor receptor precursor (EC 2.7.1.112) (PDGF-R-BETA). [Source: SWISSPROT; Acc: Q05030]	1.564
3 Glutathione reductase. [Source: RefSeq; Acc: NM_053906]	1.536
4 Cathepsin M. [Source: RefSeq; Acc: NM_181378]	1.649
5 Acetylcholine receptor protein, epsilon chain precursor. [Source: SWISSPROT; Acc: P09660]	1.535
6 Fibrinogen gamma chain precursor. [Source: SWISSPROT; Acc: P02680]	1.766
7 Protein-tyrosine phosphatase, non-receptor type 1 (EC 3.1.3.48) (protein-tyrosine phosphatase 1b) (PTP-1B). [Source: SWISSPROT; Acc: P20417]	1.530
8 Thyroglobulin precursor. [Source: SWISSPROT; Acc: P06882]	1.700
9 Deformed epidermal autoregulatory factor 1 homolog (nuclear DEAF-1-related transcriptional regulator) (NUDR) (suppressin). [Source: SWISSPROT; Acc: O88450]	1.501
10 C-CAM4 protein. [Source: RefSeq; Acc: NM_173339]	1.653
11 Glutamate receptor, ionotropic kainate 2 precursor (glutamate receptor 6) (GLUR-6) (GLUR6). [Source: SWISSPROT; Acc: P42260]	2.195
12 Syndecan-4 precursor (ryudocan core protein). [Source: SWISSPROT; Acc: P34901]	1.561
13 Insulin receptor-related protein precursor (EC 2.7.1.112) (IRR) (IR-related receptor) (fragments). [Source: SWISSPROT; Acc: Q64716]	1.781
14 BM1N (fragment). [Source: SPTREMBL; Acc: Q9QUQ2]	1.623
15 Hecpidin precursor. [Source: SWISSPROT; Acc: Q99MH3]	1.762
16 GTP cyclohydrolase I precursor (EC 3.5.4.16) (GTP-CH-I). [Source: SWISSPROT; Acc: P22288]	1.527
17 Protein-glutamine glutamyltransferase 4 (EC 2.3.2.13) (TGASE 4) (dorsal prostate transglutaminase) (dorsal protein 1) (DP1). [Source: SWISSPROT; Acc: Q99041]	1.746
18 Hepatoma-derived growth factor.	1.770
19 Oligodendrocyte transcription factor 1 (OLIGO1) (OLG-1 BHLH protein). [Source: SWISSPROT; Acc: Q9WUQ3]	1.624
20 Growth hormone receptor precursor (GH RECEPTOR) (serum binding protein). [Source: SWISSPROT; Acc: P16310]	1.522
21 Inward rectifier potassium channel 2 (potassium channel, inwardly rectifying, subfamily J, member 2) (inward rectifier K <sup>+</sup> channel KIR2.1) (RBL-IRK1). [Source: SWISSPROT; Acc: Q64273]	1.629
22 Myosin IXA. [Source: RefSeq; Acc: NM_134335]	1.550
23 Paired basic amino acid cleaving enzyme 4 precursor (EC 3.4.21.-) (subtilisin/kexin-like).	1.501
24 Fucinin 1 (fucosyltransferase inhibitor 1) (fragment). [Source: SWISSPROT; Acc: P80347]	1.547
25 Stromelysin-1 precursor (EC 3.4.24.17) (matrix metalloproteinase-3) (MMP-3) (TRANSIN-1) (SL-1) (PTR1 protein). [Source: SWISSPROT; Acc: P03957]	1.509
26 P105 coactivator. [Source: RefSeq; Acc: NM_022694]	1.752
27 TEC kinase (fragment). [Source: SPTREMBL; Acc: Q9EQ78]	1.590
28 Down syndrome critical region gene 1. [Source: RefSeq; Acc: NM_153724]	1.542
29 Ryanodine receptor type 1 (fragment). [Source: SPTREMBL; Acc: Q9R1G1]	1.674
30 Ring finger protein momo (fragment). [Source: SPTREMBL; Acc: Q8CIP0]	1.689
31 Dual-specificity protein phosphatase 6 (EC 3.1.3.48) (EC 3.1.3.16) (mitogen-activated protein kinase phosphatase 3) (MAP kinase phosphatase 3) (MKP-3). [Source: SWISSPROT; Acc: Q64346]	1.737
32 Tripartite motif protein 17; ring finger protein TERE. [Source: RefSeq; Acc: NM_022798]	1.619
33 Nuclear pore complex protein NUP88 (nucleoporin NUP88) (88 kDA nuclear pore complex protein) (nucleoporin NUP84). [Source: SWISSPROT; Acc: O08658]	1.515
34 Cytohesin 2 (ARF nucleotide-binding site opener) (ARNO protein) (CLM2) (SEC7 homolog B) (MSEC7-2). [Source: SWISSPROT; Acc: P97695]	1.530

This list was selected with Bioplot (Web site of Biopuce) upregulated threshold = 1.5,  $P < .05$  with Student's  $t$  test.

**Table W4.** Downregulated Genes by DEN Continued Underexpressed Even with CAPE Pretreatment.

Gene Name	Ratio
1 Cytochrome P450PB-1 (fragment). [Source: SPTREMBL; Acc: Q64614]	0.5942
2 Liver regeneration protein LRRYAN. [Source: RefSeq; Acc: NM_182474]	0.223
3 Adenosine kinase (EC 2.7.1.20) (AK) (adenosine 5'-phosphotransferase). [Source: SWISSPROT; Acc: Q64640]	0.635
4 Cytochrome P450 2C6 (EC 1.14.14.1) (CYPIIC6) (P450 PB1) (PTF2). [Source: SWISSPROT; Acc: P05178]	0.482
5 Cytochrome P450 2C24 (EC 1.14.14.1) (CYPIIC24) (P450-PROS2) (FRAGMENT). [Source: SWISSPROT; Acc: P33273]	0.499
6 Adenosylhomocysteinase (EC 3.3.1.1) (S-adenosyl-L-homocysteine hydrolase) (ADOHCYASE). [Source: SWISSPROT; Acc: P10760]	0.611
7 Cytochrome P450 2J3 (EC 1.14.14.1) (CYPIIJ3). [Source: SWISSPROT; Acc: P51590]	0.515
8 Meprin A $\alpha$ -subunit precursor (EC 3.4.24.18) (endopeptidase-2) (MEP-1) (endopeptidase-24.18 $\alpha$ -subunit) (E-24.18). [Source: SWISSPROT; Acc: Q64230]	0.654
9 G protein-coupled receptor 64. [Source: RefSeq; Acc: NM_181366]	0.592
10 $\alpha$ -2U globulin PGCL4. [Source: RefSeq; Acc: NM_147215]	0.443
11 Dihydropyridine-sensitive L-type, calcium channel $\beta$ -1 subunit (CAB1) (voltage-dependent calcium channel $\beta$ -1 subunit). [Source: SWISSPROT; Acc: P54283]	0.524
12 PAM COOH-terminal interactor protein 1. [Source: RefSeq; Acc: NM_022959]	0.456
13 ATP synthase oligomycin sensitivity conferral protein, mitochondrial precursor (EC 3.6.3.14) (OSCP). [Source: SWISSPROT; Acc: Q06647]	0.516
14 Major urinary protein precursor (MUP) ( $\alpha$ -2U-globulin) (15.5 kDA fatty acid binding protein) (15.5 kDA FABP) (alpha(2)-euglobulin) (allergen RAT N 1) (RAT N 1). [Source: SWISSPROT; Acc: P02761]	0.514
15 Mammary cancer-associated protein RMT-1. [Source: RefSeq; Acc: NM_145088]	0.539
16 $\alpha$ -2U globulin PGCL5. [Source: RefSeq; Acc: NM_147213]	0.531
17 Hexaprenyldihydroxybenzoate methyltransferase, mitochondrial precursor (EC 2.1.1.114) (dihydroxyhexaprenylbenzoate methyltransferase) (3,4-dihydroxy-5-hexaprenylbenzoate methyltransferase) (DHMB methyltransferase) (DHMB-MT) (DHMB-MTASE). [Source: SWISSPROT]	0.658
18 Lysophospholipase 1. [Source: RefSeq; Acc: NM_013006]	0.620
19 $\alpha$ -2U globulin. [Source: SPTREMBL; Acc: Q63221]	0.590
20 FAM3C-like protein. [Source: SPTREMBL; Acc: Q810F4]	0.609
21 Retinoblastoma-binding protein 9 (RBBP-9) (B5T overexpressed gene protein) (BOG protein). [Source: SWISSPROT; Acc: O88350]	0.442
22 Neuronal differentiation-related gene. [Source: RefSeq; Acc: NM_139333]	0.485
23 Calgranulin B (migration inhibitory factor-related protein 14) (MRP-14) (P14). [Source: SWISSPROT; Acc: P50116]	0.559
24 Cytochrome P450 2D2 (EC 1.14.14.1) (CYPIID2) (P450-DB2) (P450-CMF2) (debrisoquine 4-hydroxylase). [Source: SWISSPROT; Acc: P10634]	0.661
25 Adenomatous polyposis coli protein (APC protein). [Source: SWISSPROT; Acc: P70478]	0.431
26 CC chemokine CCL28. [Source: RefSeq; Acc: NM_053700]	0.635
27 Nuclear RNA export factor 1 (TIP associating protein) (mRNA export factor TAP). [Source: SWISSPROT; Acc: O88984]	0.598
28 Flavohemoprotein B5 + B5R. [Source: RefSeq; Acc: NM_133427]	0.625
29 Cytochrome P450 2C11 (EC 1.14.14.1) (CYPIIC11) (P-450(M-1)) (P450H) (P450-UT-A) (UT-2). [Source: SWISSPROT; Acc: P08683]	0.610
30 Vascular endothelial growth factor receptor 1 precursor (EC 2.7.1.112) (VEGFR-1) (tyrosine-protein kinase receptor FLT) (FLT-1). [Source: SWISSPROT; Acc: P53767]	0.587
31 cytochrome P450 3A1 (EC 1.14.14.1) (CYPIIA1) (P450-PCN1). [Source: SWISSPROT; Acc: P04800]	0.527
32 Testis-specific transporter TST-2. [Source: RefSeq; Acc: NM_173338]	0.665
33 $\alpha$ 2U-globulin (fragment). [Source: SPTREMBL; Acc: Q63220]	0.522
34 Ubiquitin carboxyl-terminal hydrolase L3. [Source: SPTREMBL; Acc: Q91Y78]	0.592
35 3-OXO-5-beta-steroid 4-dehydrogenase (EC 1.3.99.6) (delta(4)-3-ketosteroid 5-beta-reductase) (aldo-keto reductase family 1 member D1). [Source: SWISSPROT; Acc: P31210]	0.625
36 Serotransferrin precursor (transferrin) (siderophilin) ( $\beta$ 1-metal binding globulin). [Source: SWISSPROT; Acc: P12346]	0.445
37 RPB17 protein (fragment). [Source: SPTREMBL; Acc: Q9Z0W2]	0.596
38 GTP-binding protein RAB0. [Source: RefSeq; Acc: NM_145094]	0.562
39 Protein tyrosine phosphatase epsilon M precursor (fragment). [Source: SPTREMBL; Acc: Q63476]	0.638
40 Serum albumin precursor [contains: neurotensin-related peptide (NRP)]. [Source: SWISSPROT; Acc: P02770]	0.412
41 Sodium- and chloride-dependent GABA transporter 3. [Source: SWISSPROT; Acc: P31647]	0.551
42 Membrane protein, palmitoylated 4 (MAGUK P55 subfamily member 4). [Source: RefSeq; Acc: NM_021265]	0.599
43 Cortixin. [Source: SWISSPROT; Acc: P41237]	0.645
44 MAP-Kinase activating death domain; RAB3 GDP/GTP exchange protein. [Source: RefSeq; Acc: NM_053585]	0.645
45 Homocysteine-inducible, endoplasmic reticulum stress-inducible, ubiquitin-like domain member 1. [Source: RefSeq; Acc: NM_053523]	0.407
46 Adrenal secretory serine protease precursor. [Source: RefSeq; Acc: NM_022630]	0.613
47 Mothers against decapentaplegic homolog 1 (SMAD 1) (mothers against DPP homolog 1). [Source: SWISSPROT; Acc: P97588]	0.527
48 Cytochrome P450 2C22 (EC 1.14.14.1) (CYPIIC22) (P450 MD) (P450 P49). [Source: SWISSPROT; Acc: P19225]	0.612
49 CD80 antigen CD80 a rat homolog of the human D28/CTLA-4 ligand (B7-1). [Source: RefSeq; Acc: NM_012926]	0.615
50 RPB17 protein (fragment). [Source: SPTREMBL; Acc: Q9Z0W2]	0.615
51 Inter- $\alpha$ -trypsin inhibitor heavy chain H3 precursor (ITI heavy chain H3) (inter- $\alpha$ -inhibitor heavy chain 3). [Source: SWISSPROT; Acc: Q63416]	0.579
52 Sodium/potassium-transporting ATPase $\alpha$ -4 chain (EC 3.6.3.9) (sodium pump 4) (Na <sup>+</sup> /K <sup>+</sup> ATPase 4). [Source: SWISSPROT; Acc: Q64541]	0.607
53 Phospholipid scramblase 1 (PL scramblase 1) (Ca(2+)-dependent phospholipid scramblase 1). [Source: SWISSPROT; Acc: P58195]	0.547
54 Cytochrome P450 2C12, female-specific (EC 1.14.14.1) (CYPIIC12) (P450I) (15-BETA) (P450-UT-I) (UT-1). [Source: SWISSPROT; Acc: P11510]	0.571
55 Mast cell protease I precursor (EC 3.4.21.39) (RMCP-1) (CHYMASE). [Source: SWISSPROT; Acc: P09650]	0.488
56 Vav proto-oncogene (P95). [Source: SWISSPROT; Acc: P54100]	0.635

This list was selected with Bioplot (Web site of Biopuce) downregulated threshold = 0.66,  $P < .05$  with Student's  $t$  test.

**Table W5.** Downregulated Genes by DEN Restored to Profile Level of Non Treated Rats with CAPE Pretreatment.

Gene Name	Ratio
1 Cytochrome B5. [Source: SWISSPROT; Acc: P00173]	0.633
2 Cytochrome P450 3A18 (EC 1.14.14.1) (CYP11A18) (P450(6)beta-2). [Source: SWISSPROT; Acc: Q64581]	0.618
3 Hydroxymethylglutaryl-CoA synthase, mitochondrial precursor (EC 2.3.3.10) (HMG-CoA synthase) (3-hydroxy-3-methylglutaryl coenzyme A synthase). [Source: SWISSPROT; Acc: P22791]	0.647
4 Acyl-CoA desaturase (EC 1.14.19.1) (stearoyl-CoA desaturase) (fatty acid desaturase) (delta(9)-desaturase). [Source: SWISSPROT; Acc: P07308]	0.657
5 Vesicular glutamate transporter 3. [Source: RefSeq; Acc: NM_153725]	0.620
6 Cytochrome P450 2C13, male-specific (EC 1.14.14.1) (CYP11C13) (P450-G) (UT-5). [Source: SWISSPROT; Acc: P20814]	0.660
7 Putative RNA binding protein 1 (fragment). [Source: SPTREMBL; Acc: O08882]	0.646
8 Cyclin-dependent kinase inhibitor 2A (P16, INHIBITS CDK4). [Source: RefSeq; Acc: NM_031550]	0.662
9 Phosphorylase B kinase gamma catalytic chain, testis/liver isoform (EC 2.7.1.38) (PHK-GAMMA-T) (phosphorylase kinase gamma subunit 2). [Source: SWISSPROT; Acc: P31325]	0.661
10 Carbonic anhydrase III (EC 4.2.1.1) (carbonate dehydratase III) (CA-III). [Source: SWISSPROT; Acc: P14141]	0.658
11 $\alpha$ -2-Macroglobulin precursor ( $\alpha$ -2-M). [Source: SWISSPROT; Acc: P06238]	0.641
12 Anion exchange protein 2 (non-erythroid band 3-like protein) (B3RP). [Source: SWISSPROT; Acc: P23347]	0.655
13 Calpain 8. [Source: RefSeq; Acc: NM_133309]	0.655

This list was selected with Bioplot (Web site of Biopuce) downregulated threshold = 0.66,  $P < .05$  with Student's  $t$  test.



**Table W6.** Downregulated Genes Only When There Is CAPE Pretreatment before DEN.

Gene Name	Ratio
1 Cytochrome P450, 1A2; cytochrome P450, subfamily I (aromatic compound-inducible), member A2 (Q42, FORM D). [Source: RefSeq; Acc: NM_012541]	0.577
2 Proton-associated sugar transporter A. [Source: RefSeq; Acc: NM_144747]	0.560
3 MHC class I protein precursor. [Source: SPTREMBL; Acc: Q9JHM2]	0.599
4 Ferritin light chain (ferritin L subunit). [Source: SWISSPROT; Acc: P02793]	0.592
5 Pregnancy-zone protein. [Source: RefSeq; Acc: NM_145779]	0.591
6 Excitatory amino acid transporter 3 (sodium-dependent glutamate/aspartate transporter 3) (excitatory amino acid carrier 1). [Source: SWISSPROT; Acc: P51907]	0.478
7 Indoleamine 2,3-dioxygenase. [Source: RefSeq; Acc: NM_023973]	0.583
8 BM1N (fragment). [Source: SPTREMBL; Acc: Q9QUQ2]	0.492
9 Diacylglycerol kinase, beta (EC 2.7.1.107) (diglyceride kinase) (DGK-BETA) (DAG kinase beta) (90 kDa diacylglycerol kinase). [Source: SWISSPROT; Acc: P49621]	0.656
10 Vitamin D-binding protein precursor (DBP) (group-specific component) (GC-globulin) (VDB). [Source: SWISSPROT; Acc: P04276]	0.608
11 Period homolog 3. [Source: RefSeq; Acc: NM_023978]	0.618
12 Leucine-rich glioma-inactivated protein 1 precursor. [Source: SWISSPROT; Acc: Q8K4Y5]	0.659
13 MSX-interacting-zinc finger. [Source: RefSeq; Acc: NM_053337]	0.426
14 Clock gene. [Source: RefSeq; Acc: NM_021856]	0.460
15 Sodium-dependent neutral amino acid transporter ASCT2. [Source: RefSeq; Acc: NM_175758]	0.607
16 NK cell lectin-like receptor family E, member 1. [Source: RefSeq; Acc: NM_181372]	0.617
17 DREBRIN (developmentally regulated brain protein). [Source: SWISSPROT; Acc: Q07266]	0.472
18 Sperm autoantigenic protein 17. [Source: RefSeq; Acc: NM_053482]	0.576
19 Leucine-rich repeat-containing protein 15 precursor (RLIB). [Source: SWISSPROT; Acc: Q8R5M3]	0.533
20 Lamin A. [Source: SWISSPROT; Acc: P48679]	0.524
21 Galactoside 2- $\alpha$ -L-fucosyltransferase 2 (EC 2.4.1.69) (secretor blood group $\alpha$ -2-fucosyltransferase) (GDP-L-fucose- $\beta$ -D-galactoside 2- $\alpha$ -L-fucosyltransferase 2) ( $\alpha$ (1,2)FT 2) (fucosyltransferase 2) (fragment). [Source: SWISSPROT; Acc: Q10984]	0.569
22 RN protein. [Source: RefSeq; Acc: NM_133585]	0.551
23 Mitogen-activated protein kinase 14 (EC 2.7.1.37) (mitogen-activated protein kinase p38 $\alpha$ ) (MAP kinase p38 $\alpha$ ). [Source: SWISSPROT; Acc: P70618]	0.631
24 Trypsin III, cationic precursor (EC 3.4.21.4) (pretrypsinogen III). [Source: SWISSPROT; Acc: P08426]	0.341
25 Glycogenin-1 (EC 2.4.1.186). [Source: SWISSPROT; Acc: O08730]	0.551
26 Transcription termination factor, mitochondrial. [Source: RefSeq; Acc: NM_053499]	0.644
27 Tyrosine-protein kinase CSK (EC 2.7.1.112) (C-SRC kinase). [Source: SWISSPROT; Acc: P32577]	0.518
28 Neurturin. [Source: SPTREMBL; Acc: Q811Q5]	0.524
29 Cellular tumor antigen p53 (tumor suppressor p53). [Source: SWISSPROT; Acc: P10361]	0.509
30 Partitioning-defective 3 homolog (PARD-3) (PAR-3) (atypical PKC isotype-specific interacting protein) (ASIP) (atypical PKC specific binding protein) (ASBP). [Source: SWISSPROT; Acc: Q9Z340]	0.655
31 Small inducible cytokine A5 precursor (CCL5) (T-cell-specific rantes protein) (SIS-delta). [Source: SWISSPROT; Acc: P50231]	0.568
32 CDIG1L protein. [Source: RefSeq; Acc: NM_153623]	0.644
33 RT1 class IB gene, H2-TL-like, GRC region. [Source: RefSeq; Acc: NM_012646]	0.601
34 Alcohol sulfotransferase A (EC 2.8.2.2) (hydroxysteroid sulfotransferase A) (STA) (androsterone-sulfating sulfotransferase) (AD-ST) (ST-40). [Source: SWISSPROT; Acc: P22789]	0.615
35 RHO-interacting protein 3 (P116RIP) (RIP3). [Source: SWISSPROT; Acc: Q9ERE6]	0.569
36 Heat shock protein, $\beta$ -2 (HSPB2). [Source: SWISSPROT; Acc: O35878]	0.650
37 Transcription factor SOX-10. [Source: SWISSPROT; Acc: O55170]	0.529
38 Cation chloride cotransporter 9. [Source: RefSeq; Acc: NM_153625]	0.665
39 N-ethylmaleimide-sensitive factor. [Source: RefSeq; Acc: NM_021748]	0.472
40 Cytochrome c oxidase subunit IV isoform 2, mitochondrial precursor (EC 1.9.3.1) (COX IV-2). [Source: SWISSPROT; Acc: Q91Y94]	0.646
41 Serotonin N-acetyltransferase (EC 2.3.1.87) (aralkylamine N-acetyltransferase) (AA-NAT) (serotonin acetylase). [Source: SWISSPROT; Acc: Q64666]	0.582
42 Sterol O-acyltransferase 1 (EC 2.3.1.26) (cholesterol acyltransferase 1) (acyl coenzyme A:cholesterol acyltransferase 1) (ACAT-1). [Source: SWISSPROT; Acc: O70536]	0.593
43 Calcium-activated potassium channel $\beta$ 2. [Source: RefSeq; Acc: NM_176861]	0.479
44 Outer dense fiber protein (RT7 protein) (RTS 5/1). [Source: SWISSPROT; Acc: P21769]	0.602
45 Contrapsin-like protease inhibitor 1 precursor (CPI-21) (kallikrein-binding protein) (KBP) (growth hormone-regulated proteinase inhibitor) (serine protease inhibitor 2) (SPI-2) (GHR-P63) (SPI-2.3) (thyroid hormone-regulated protein). [Source: SWISSPROT; Acc: P05545]	0.655
46 Biglycan precursor (bone/cartilage proteoglycan I) (PG-S1). [Source: SWISSPROT; Acc: P47853]	0.519
47 Doublesex and MAB-3 related transcription factor 1. [Source: RefSeq; Acc: NM_053706]	0.580
48 Fatty acid-binding protein, brain (B-FABP) (brain lipid-binding protein) (BLBP). [Source: SWISSPROT; Acc: P55051]	0.487
49 von Willebrand factor vWF (von Willibrand factor) (fragment). [Source: SPTREMBL; Acc: Q62935]	0.656
50 Maspin precursor (protease inhibitor 5). [Source: SWISSPROT; Acc: P70564]	0.629
51 Receptor-activity modifying protein 1. [Source: RefSeq; Acc: NM_031645]	0.654
52 Transketolase (EC 2.2.1.1) (TK). [Source: SWISSPROT; Acc: P50137]	0.581
53 Transcription factor E2F6 (fragment). [Source: SPTREMBL; Acc: Q80ZB0]	0.624
54 Golgi autoantigen, golgin subfamily A member 2 ( <i>cis</i> -Golgi matrix protein GM130). [Source: SWISSPROT; Acc: Q62839]	0.630
55 Dynein-like protein 5 (fragment). [Source: SPTREMBL; Acc: Q63168]	0.614
56 DNA polymerase gamma subunit 1 (EC 2.7.7.7) (mitochondrial dna polymerase catalytic subunit) (POLG-alpha). [Source: SWISSPROT; Acc: Q9QYV8]	0.528
57 Keratinocyte growth factor precursor (KGF) (fibroblast growth factor-7) (FGF-7) (HBGF-7). [Source: SWISSPROT; Acc: Q02195]	0.559
58 ferm-domain-containing protein 163SCII (fragment). [Source: SPTREMBL; Acc: Q8V8II]	0.662
59 Titin (fragment). [Source: SPTREMBL; Acc: Q9JJ49]	0.618
60 Carbonic anhydrase IV precursor (EC 4.2.1.1) (carbonate dehydratase IV) (CA-IV). [Source: SWISSPROT; Acc: P48284]	0.635
61 DNA polymerase delta catalytic subunit (EC 2.7.7.7). [Source: SWISSPROT; Acc: O54747]	0.627
62 Serine/threonine-protein kinase PAK 3 (EC 2.7.1.-) (P21-activated kinase 3) (PAK-3) (beta-PAK) (P65-PAK). [Source: SWISSPROT; Acc: Q62829]	0.586
63 Noggin precursor (fragment). [Source: SWISSPROT; Acc: Q62809]	0.663
64 Sphingosine kinase 1. [Source: RefSeq; Acc: NM_133386]	0.586
65 Tyrosine 3-monooxygenase (EC 1.14.16.2) (tyrosine 3-hydroxylase) (TH). [Source: SWISSPROT; Acc: P04177]	0.662
66 Protein kinase C, epsilon type (EC 2.7.1.-) (NPKC-epsilon). [Source: SWISSPROT; Acc: P09216]	0.626
67 Retinal pigment epithelium, 65 kDa. [Source: RefSeq; Acc: NM_053562]	0.554
68 Cytochrome c oxidase polypeptide via - heart, mitochondrial precursor (EC 1.9.3.1) (COXVIAH) (fragment). [Source: SWISSPROT; Acc: P10817]	0.595
69 $\beta$ -Arrestin 1 (arrestin, beta 1). [Source: SWISSPROT; Acc: P29066]	0.513
70 Neurogenic locus notch homolog protein 2 precursor (NOTCH 2). [Source: SWISSPROT; Acc: Q9QW30]	0.626
71 NADH dehydrogenase (ubiquinone) 1 alpha subcomplex 10. [Source: RefSeq; Acc: NM_182671]	0.545

Table W6. (continued).

	Gene Name	Ratio
72	Cyclin-dependent kinase inhibitor 2C; cyclin-dependent kinase inhibitor 2C (P18, inhibits CDK4), see also D5LEV19. [Source: RefSeq; Acc: NM_131902]	0.544
73	Neural cell adhesion molecule 1, 140 kDa isoform precursor (N-CAM 140) (NCAM-140). [Source: SWISSPROT; Acc: P13596]	0.578
74	Dihydropyrimidinase (EC 3.5.2.2) (DHPASE) (hydantoinase) (DHP). [Source: SWISSPROT; Acc: Q63150]	0.653
75	Phosphoenolpyruvate carboxykinase, cytosolic [GTP] (EC 4.1.1.32) (phosphoenolpyruvate carboxylase) (PEPCK-C). [Source: SWISSPROT; Acc: P07379]	0.550
76	Probable G protein-coupled receptor GPR19 (fragment). [Source: SWISSPROT; Acc: P70585]	0.414
77	Homeobox protein MSX-2 (HOX-8.1) (fragment). [Source: SWISSPROT; Acc: P52953]	0.547
78	Potassium voltage-gated channel subfamily KQT member 2 (potassium channel $\alpha$ subunit KVLQT2) (KQT-LIKE 2). [Source: SWISSPROT; Acc: O88943]	0.649
79	Camello-like 1. [Source: RefSeq; Acc: NM_133558]	0.570
80	Adenylate cyclase, type II (EC 4.6.1.1) (ATP pyrophosphate-lyase) (adenylyl cyclase). [Source: SWISSPROT; Acc: P26769]	0.624
81	Immunoglobulin-binding protein 1 (CD79A-binding protein 1) ( $\alpha_4$ phosphoprotein). [Source: SWISSPROT; Acc: O08836]	0.637
82	Proteasome subunit $\alpha$ type 7 (EC 3.4.25.1) (proteasome subunit RC6-1). [Source: SWISSPROT; Acc: P48004]	0.607
83	ADAM 7 precursor (a disintegrin and metalloproteinase domain 7) (epididymal apical protein I) (EAP I). [Source: SWISSPROT; Acc: Q63180]	0.468
84	KIAA1454-like protein (fragment). [Source: SPTREMBL; Acc: Q99MF6]	0.663
85	$\alpha$ -Lactalbumin precursor (lactose synthase B protein). [Source: SWISSPROT; Acc: P00714]	0.647
86	Flavin-containing monooxygenase 2. [Source: RefSeq; Acc: NM_144737]	0.559
87	Aggrecan core protein precursor (cartilage-specific proteoglycan core protein) (CSPCP). [Source: SWISSPROT; Acc: P07897]	0.639
88	Dihydrofolate reductase; dihydrofolate reductase 1 (active). [Source: RefSeq; Acc: NM_130400]	0.530
89	Max-interacting protein 1 (MXI1 protein). [Source: SWISSPROT; Acc: O09015]	0.526
90	gamma Crystallin E (gamma crystallin 3-1) (gamma-2). [Source: SWISSPROT; Acc: P02528]	0.455
91	heat shock-related 70 kDa protein 2 (heat shock protein 70.2) (testis-specific heat shock protein-related) (HST). [Source: SWISSPROT; Acc: P14659]	0.466
92	Synaptobrevin-like 1. [Source: RefSeq; Acc: NM_053531]	0.605
93	DNA-binding protein A (cold shock domain protein A) (muscle Y-BOX protein YB2) (Y-BOX binding protein-A) (RYB-A). [Source: SWISSPROT; Acc: Q62764]	0.595
94	Proprotein convertase subtilisin/kexin type 1 inhibitor; granin-like neuroendocrine peptide precursor. [Source: RefSeq; Acc: NM_019279]	0.664
95	Vitronectin. [Source: RefSeq; Acc: NM_019156]	0.550
96	CYS2/HIS2 zinc finger protein (RKR1). [Source: RefSeq; Acc: NM_144757]	0.617
97	Urea transporter, kidney. [Source: SWISSPROT; Acc: Q62668]	0.616
98	Urinary protein 3 precursor (RUP-3). [Source: SWISSPROT; Acc: P83121]	0.655
99	Carcinoembryonic antigen-related protein (fragment). [Source: SPTREMBL; Acc: Q63112]	0.640
100	Growth arrest specific 6. [Source: RefSeq; Acc: NM_057100]	0.552
101	Leptin receptor gene-related protein (OB-R gene-related protein) (OB-RGRP). [Source: SWISSPROT; Acc: Q9JLS8]	0.620
102	G protein-coupled receptor 9. [Source: RefSeq; Acc: NM_053415]	0.659
103	Voltage-dependent t-type calcium channel $\alpha$ -1H subunit (CAV3.2). [Source: SWISSPROT; Acc: Q9EQ60]	0.597
104	Voltage-gated potassium channel subfamily H member 5 (ether-A-GO-GO potassium channel 2) (REAG2). [Source: SWISSPROT; Acc: Q9EPI9]	0.498
105	L-Serine dehydratase/L-threonine deaminase [includes: L-serine dehydratase (EC 4.3.1.17) (L-serine deaminase) (SDH); L-threonine dehydratase (EC 4.3.1.19) (L-threonine deaminase) (TDH)]. [Source: SWISSPROT; Acc: P09367]	0.486
106	Sodium- and chloride-dependent glycine transporter 1 (GLYT1) (GLYT-1). [Source: SWISSPROT; Acc: P28572]	0.616
107	Platelet endothelial tetraspan antigen 3 (CD151 antigen). [Source: SWISSPROT; Acc: Q9QZA6]	0.572
108	YY1 transcription factor. [Source: RefSeq; Acc: NM_173290]	0.608
109	Phosphoinositid 3-phosphate-binding protein-2-like protein. [Source: SPTREMBL; Acc: Q8K481]	0.432
110	Protein phosphatase-X (EC 3.1.3.16) (serine/threonine protein phosphatase) (fragment). [Source: SPTREMBL; Acc: Q04104]	0.596
111	Visinin-like protein 2 (VILIP-2) (neural visinin-like protein 2) (NVL-2) (NVP-2). [Source: SWISSPROT; Acc: P35332]	0.622
112	Cytochrome P450 2E1 (EC 1.14.14.1) (CYPIIE1) (P450-J) (P450RLM6). [Source: SWISSPROT; Acc: P05182]	0.595
113	Gap junction channel protein connexin47 (fragment). [Source: SPTREMBL; Acc: Q80XF7]	0.604
114	Zinc finger protein 384 (nuclear matrix transcription factor 4) (CAS-associated zinc finger protein). [Source: SWISSPROT; Acc: Q9EQJ4]	0.652
115	Huntingtin-associated protein-interacting protein (duo protein) (kalinin) (PAM COOH-terminal interactor protein 10) (P-CIP10). [Source: SWISSPROT; Acc: P97924]	0.562
116	Actinfilin. [Source: RefSeq; Acc: NM_145671]	0.517
117	Glutamate receptor, ionotropic kainate 5 precursor (glutamate receptor KA-2) (KA2). [Source: SWISSPROT; Acc: Q63273]	0.627
118	Glutaminase, kidney isoform, mitochondrial precursor (EC 3.5.1.2) (GLS) (L-glutamine amidohydrolase) (K-glutaminase). [Source: SWISSPROT; Acc: P13264]	0.658
119	Guanine nucleotide-binding protein G(OLF), $\alpha$ subunit (adenylate cyclase-stimulating G $\alpha$ protein, olfactory type). [Source: SWISSPROT; Acc: P38406]	0.597
120	Chloride intracellular channel protein 5. [Source: SWISSPROT; Acc: Q9EPT8]	0.418
121	Prolyl endopeptidase. [Source: RefSeq; Acc: NM_031324]	0.438
122	RAB6-interacting protein 2; RIM-binding protein; ELKS. [Source: RefSeq; Acc: NM_170788]	0.590
123	Dynein-like protein 10 (fragment). [Source: SPTREMBL; Acc: Q63161]	0.392
124	Thymidine kinase, cytosolic (EC 2.7.1.21) (fragment). [Source: SWISSPROT; Acc: P27158]	0.660
125	Zinc finger and homeodomain protein 1. [Source: RefSeq; Acc: NM_133620]	0.459
126	Regulator of G-protein signaling 6 (RGS6) (fragment). [Source: SWISSPROT; Acc: P49801]	0.591
127	Chondroitin sulfate proteoglycan NG2 precursor (HSN tumor-specific antigen). [Source: SWISSPROT; Acc: Q00657]	0.577
128	Epimorphin (syntaxin 2). [Source: SWISSPROT; Acc: P50279]	0.492
129	$\alpha$ -1D Adrenergic receptor ( $\alpha$ 1d-adrenoceptor) ( $\alpha$ -1a adrenergic receptor) (RA42). [Source: SWISSPROT; Acc: P23944]	0.623
130	Enoyl-CoA hydratase, mitochondrial precursor (EC 4.2.1.17) (short chain enoyl-CoA hydratase) (SCEH) (enoyl-CoA hydratase 1). [Source: SWISSPROT; Acc: P14604]	0.458
131	RAT AXL shortform. [Source: SPTREMBL; Acc: Q8V199]	0.658
132	Calcium/calmodulin-dependent protein kinase II subtype delta 5 (fragment). [Source: SPTREMBL; Acc: Q63907]	0.630
133	Valosin-containing protein (P97)/P47 complex-interacting protein P135; VCP(P97)/P47-interacting protein. [Source: RefSeq; Acc: NM_176857]	0.541
134	Glutathione S-transferase YRS-YRS (EC 2.5.1.18) (GST 12-12) (glutathione S-transferase subunit 12) (GST class-THETA). [Source: SWISSPROT; Acc: P30713]	0.659
135	6-Phosphofructokinase, type C (EC 2.7.1.11) (phosphofructokinase 1) (phosphohexokinase) (phosphofructo-1-kinase isozyme C) (PFK-C) (fragment). [Source: SWISSPROT; Acc: P47860]	0.570
136	GDNF family receptor $\alpha_4$ precursor (GFR- $\alpha_4$ ) (GFRALPHA4) (persephin receptor). [Source: SWISSPROT; Acc: Q9EPI2]	0.558
137	Heterogeneous nuclear ribonucleoprotein R. [Source: RefSeq; Acc: NM_175603]	0.635
138	Membrane glycoprotein. [Source: RefSeq; Acc: NM_020081]	0.488
139	Nuclear factor 1 A-type (nuclear factor 1/A) (NF1-A) (NF-I/A) (CCAAT-BOX binding transcription factor) (CTF) (TGGCA-binding protein). [Source: SWISSPROT; Acc: P09414]	0.519
140	Monocyte differentiation antigen CD14 precursor (myeloid cell-specific leucine-rich glycoprotein). [Source: SWISSPROT; Acc: Q63691]	0.563
141	Cytochrome P450 2A2 (EC 1.14.14.1) (CYPIIA2) (testosterone 15- $\alpha$ -hydroxylase) (P450-UT-4). [Source: SWISSPROT; Acc: P15149]	0.485
142	Neuronal acetylcholine receptor protein, $\alpha$ -2 chain precursor. [Source: SWISSPROT; Acc: P12389]	0.639

Table W6. (continued).

Gene Name	Ratio
143 Augmenter of liver regeneration. [Source: SWISSPROT; Acc: Q63042]	0.421
144 ATP-binding cassette, subfamily C (CFTR/MRP), member 3. [Source: RefSeq; Acc: NM_080581]	0.512
145 Yotiao protein (fragment). [Source: SPTREMBL; Acc: Q9JHE0]	0.637
146 Striatin. [Source: SWISSPROT; Acc: P70483]	0.574
147 Plasminogen activator inhibitor-2, type A (PAI2A) (PAI-2). [Source: SWISSPROT; Acc: P29524]	0.502
148 Forkhead box protein G1A (forkhead-related protein FKHL2) (transcription factor BF-2) (brain factor 2) (BF2) (fragment). [Source: SWISSPROT; Acc: Q63251]	0.527
149 $\alpha$ -2U globulin PGCL3. [Source: RefSeq; Acc: NM_147212]	0.522
150 Acyl coenzyme A thioester hydrolase, mitochondrial precursor (EC 3.1.2.2) (very-long-chain acyl-CoA thioesterase) (MTE-I). [Source: SWISSPROT; Acc: O55171]	0.630
151 Thioredoxin-like (32KD). [Source: RefSeq; Acc: NM_080887]	0.638
152 cAMP-dependent protein kinase, $\alpha$ -catalytic subunit (EC 2.7.1.37) (PKA C- $\alpha$ ). [Source: SWISSPROT; Acc: P27791]	0.651
153 40S ribosomal protein S3A (V-fos transformation effector protein). [Source: SWISSPROT; Acc: P49242]	0.597
154 Rabphilin-3A (exophilin 1). [Source: SWISSPROT; Acc: P47709]	0.664
155 Neogenin precursor (fragment). [Source: SWISSPROT; Acc: P97603]	0.520
156 Degenerative spermatocyte homolog. [Source: RefSeq; Acc: NM_053323]	0.346
157 sirtuin 2 (silent mating type information regulation 2, homolog) 2; 5E5 antigen. [Source: RefSeq; Acc: NM_053737]	0.564
158 Nuclear GTPase pike. [Source: RefSeq; Acc: NM_023026]	0.503
159 Mucin 1 (fragment). [Source: SPTREMBL; Acc: O35770]	0.551
160 Protease, serine, 12; protease, serine, 12 neurotrypsin (motopsin). [Source: RefSeq; Acc: NM_053504]	0.620
161 C-ETS-1 protein (P54). [Source: SWISSPROT; Acc: P41156]	0.591
162 VIP-receptor transcriptional repressor protein (VIPR-RP). [Source: SPTREMBL; Acc: O88461]	0.642
163 1-Phosphatidylinositol-4,5-bisphosphate phosphodiesterase gamma 1 (EC 3.1.4.11) (phosphoinositide phospholipase C) (PLC-gamma-1) (phospholipase C-gamma-1) (PLC-II) (PLC-148). [Source: SWISSPROT; Acc: P10686]	0.596
164 ATP-binding cassette, subfamily A (ABC1), member 5. [Source: RefSeq; Acc: NM_173307]	0.608
165 Interleukin-1 beta precursor (IL-1 beta). [Source: SWISSPROT; Acc: Q63264]	0.664
166 Forkhead box protein C2 (brain factor-3) (BF-3) (HFFH-BF-3) (fragment). [Source: SWISSPROT; Acc: Q63246]	0.580
167 $\alpha$ -2 Antiplasmin; pigment epithelium-derived factor. [Source: RefSeq; Acc: NM_177927]	0.452
168 Aldehyde dehydrogenase 1A2 (EC 1.2.1.3) (retinaldehyde-specific dehydrogenase type 2) (RALDH(II)) (RALDH-2). [Source: SWISSPROT; Acc: Q63639]	0.622
169 Solute carrier family 38, member 2; amino acid transporter, cationic 2 (low affinity); amino acid transporter system A2. [Source: RefSeq; Acc: NM_181090]	0.622
170 Glial cell line-derived neurotrophic factor precursor. [Source: SWISSPROT; Acc: Q07731]	0.652
171 Receptor tyrosine kinase. [Source: SPTREMBL; Acc: Q9EPA1]	0.577
172 Serine/threonine protein phosphatase 2A, 55-kDA regulatory subunit B, $\alpha$ isoform (PP2A, subunit B, B-alpha isoform) (PP2A, subunit B, B55-alpha isoform) (PP2A, subunit B, PR55-alpha isoform) (PP2A, subunit B, R2-alpha isoform) [Source: SWISSPROT; Acc: P36876]	0.611
173 Vesicle-associated membrane protein 8 (endobrevin). [Source: RefSeq; Acc: NM_031827]	0.518
174 Claudin-7 (fragment). [Source: SWISSPROT; Acc: Q9Z1L1]	0.643
175 XRCC5 (fragment). [Source: SPTREMBL; Acc: Q8VIB0]	0.618
176 cAMP-dependent protein kinase type II-alpha regulatory chain (fragment). [Source: SWISSPROT; Acc: P12368]	0.631
177 GTP-binding protein REM2; ras-related GTP-binding protein of the RAD/GEM/KIR family; ras-related GTP-binding protein of the RAD/GEM/KIR family, member 2. [Source: RefSeq; Acc: NM_022685]	0.578
178 40S ribosomal protein S9. [Source: SWISSPROT; Acc: P29314]	0.660
179 Histone H2A, testis. [Source: SWISSPROT; Acc: Q00728]	0.519
180 Voltage-dependent P/Q-type calcium channel $\alpha$ -1A subunit (calcium channel, L type, $\alpha$ -1 polypeptide, isoform 4) (brain calcium channel I) (BI) (rat brain class A) (RBA-I). [Source: SWISSPROT; Acc: P54282]	0.559
181 Cathepsin K precursor (EC 3.4.22.38). [Source: SWISSPROT; Acc: O35186]	0.548
182 Werner syndrome homolog (human) interacting protein. [Source: RefSeq; Acc: NM_172332]	0.619
183 NADP-dependent malic enzyme (EC 1.1.1.40) (NADP-ME) (malic enzyme 1). [Source: SWISSPROT; Acc: P13697]	0.637
184 Dihydropyrimidinase related protein-1 (DRP-1) (collapsin response mediator protein 1) (CRMP-1). [Source: SWISSPROT; Acc: Q62950]	0.586
185 Heat shock 27 kDa protein (HSP 27). [Source: SWISSPROT; Acc: P42930]	0.539
186 Zinc finger protein 94 (ZFP-94) (zinc finger protein Y1) (RLZF-Y). [Source: SWISSPROT; Acc: Q9Z2K3]	0.579
187 Cytochrome P450 2B1 (EC 1.14.14.1) (CYP11B1) (P450-B) (P450-PB1 and P450-PB2) (P450-LM2). [Source: SWISSPROT; Acc: P00176]	0.657

This list was selected with Bioplot (Web site of Biopuce) downregulated threshold = 0.66,  $P < .05$  with Student's  $t$  test.