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# An Approach to the Study of Gene Expression in Hepatocarcinogenesis Initiation[1,2]

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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.

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## 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 Bioplot, 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) |
|---|---|---|---|---|
| *CYP1A2* | NM_012541 | AGGGACACCTCACTGAATGG | CCGAAGAGCATCACCTTCTC | 182 |
| *CYP2A2* | NM_012693 | ATCCAGATGTGGAAGCCAAG | CCACGGAAGGTTGTGTTCTT | 187 |
| *CYP2B1* | NM_001134844 | GGAAGCTCTGGTTGCTTGAC | CAAAGAAGTGCAGACCGACA | 206 |
| *CYP2E1* | NM_031543 | CCTACATGGATGCTGTGGTG | CTGGAAACTCATGGCTGTCA | 171 |
| *GST-θ* | NM_012796 | ATGGCATTCCCTTTCAGTTG | GTGGTCTGCCACCTGGTACT | 179 |
| *GST-κ* | NM_181371 | CACGGAGTCCCAGAACATTT | CGGTCAGACCCAAATAGCAT | 210 |
| *p53* | NM_030989 | TCTCCCAGCAAAAGAAAAA | CTTCGGGTAGCTGGAGTGAG | 168 |
| *p38* | NM_031020 | AGCGATACCAGAACCTGTCC | GAACGTGGTCATCGGTAAGC | 318 |
| *YY1* | NM_173290 | GACGACGACTACATCGAGCA | TTGATCTGCACCTGCTTCTG | 209 |
| *DNApolδ* | NM_021662 | GACGACGACTACATCGAGCA | AGCGGGAGGAGAAGAGTAGG | 217 |
| *GLUTATHION REDUCTASE* | NM_053906 | CCATGTGGTTACTGCACTTCC | GTTCCTTTCCTTCTCCTGAGC | 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.

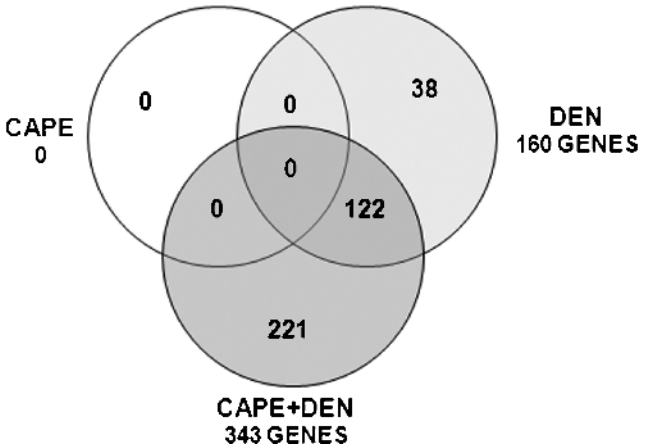


**Figure 1.** Venn diagram of transcriptionally altered genes. Data were analyzed using the Venn diagram program from http://biopuce.insa-toulouse.fr. Genes were identified as upregulated (threshold > 1.5) or downregulated (threshold < 0.6) with $P < .05$ considered statistically significant with Student's *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*

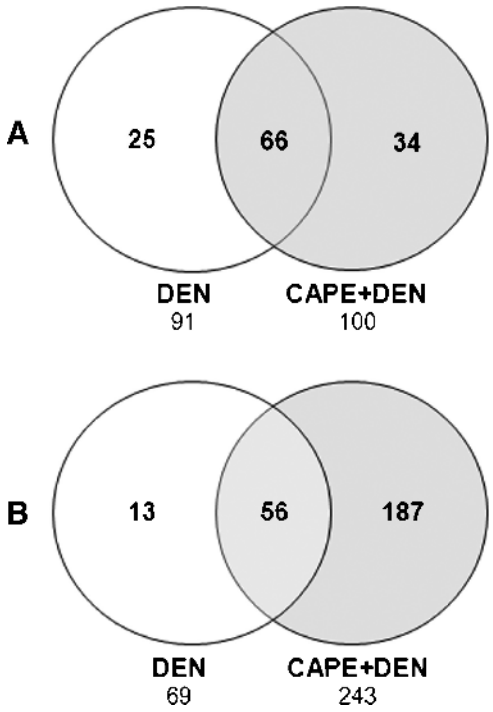


**Figure 2.** Comparison of Venn diagrams for genes upregulated or downregulated after DEN or DEN plus CAPE treatment. (A) Upregulated genes, threshold greater than 1.5 with $P < .05$ by Student's *t* test. (B) Downregulated genes, threshold less than 0.6 with $P < .05$ by Student's *t* test. Data were 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 |
|---|---|
| *CYP2B1** | |
| *CYP1A2** | DEN metabolism |
| *CYP2A2** | |
| *CYP2E1* | |
| *GST-κ*† | |
| *GST-θ** | Oxidative stress regulation |
| *GLUTATIÓN REDUCTASE** | |
| *YY1** | |
| *DNA polδ** | Replication |
| *p53** | DNA reparation |
| *p38** | Cell signaling process. |

* 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 2*A*).

### *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*, *macroglobulins*, *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 downregulated 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 2*B*).

### *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

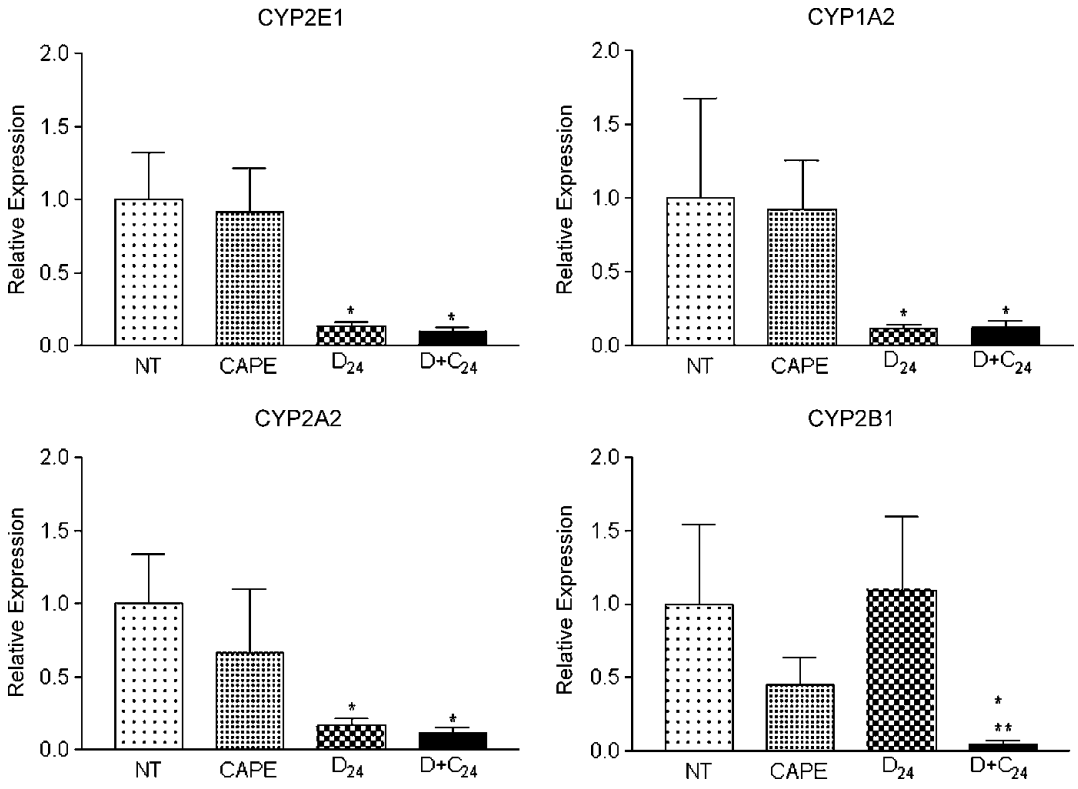


**Figure 3.** RT-PCR validation of *CYP* expression levels. Samples were analyzed by RT-PCR using primers described in Table 1. *CAPE* indicates rats killed 12 hours after CAPE dosing; $D + C_{24}$, killed 24 hours after DEN dosing with CAPE pretreatment 12 hours before DEN ($n = 4$ in each group); $D_{24}$, killed 24 hours after DEN dosing; *NT*, untreated. $^*P < .05$ compared with NT, $^{**}P < .05$ compared with $D_{24}$ 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.

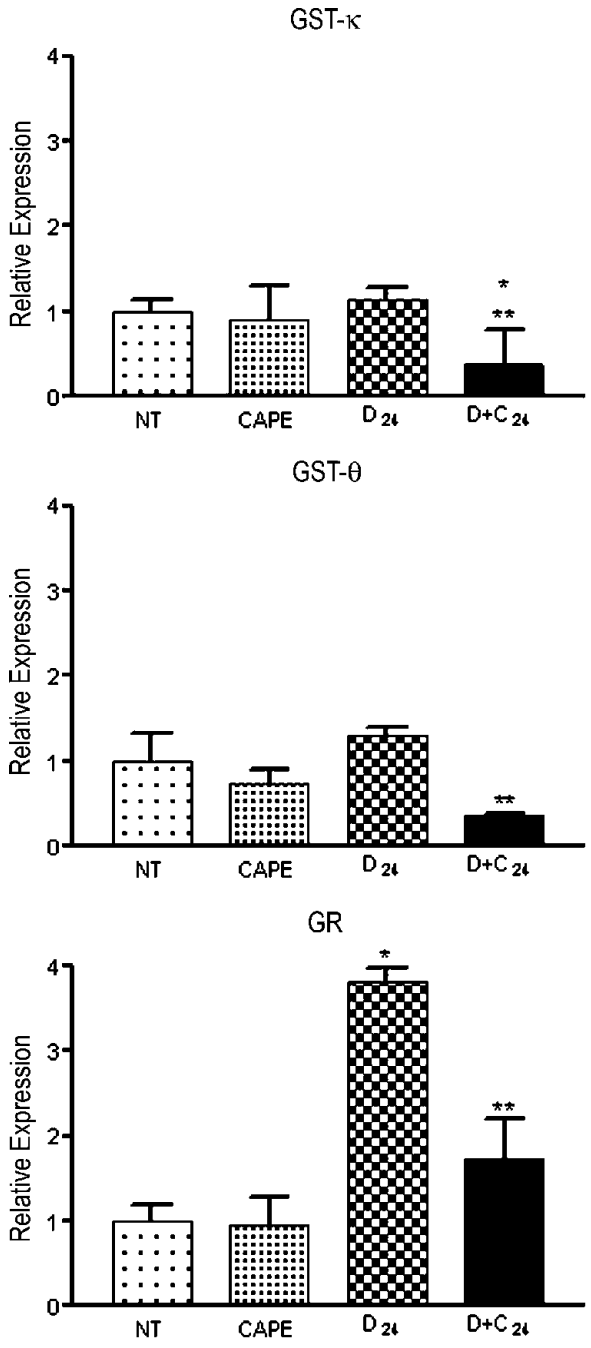


**Figure 4.** RT-PCR validation of *GST-κ*, *GST-θ*, and *glutathione reductase* (*GR*) expression levels. Samples were analyzed by RT-PCR using primers from Table 1. *CAPE* indicates rats killed 12 hours after CAPE dosing; $D + C_{24}$, killed 24 hours after DEN dosing with CAPE pretreatment 12 hours before DEN ($n = 4$ in each group); $D_{24}$, killed 24 hours after DEN dosing; *NT*, untreated. $^{*}P < .05$ compared with NT, $^{**}P < .05$ compared with $D_{24}$ 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

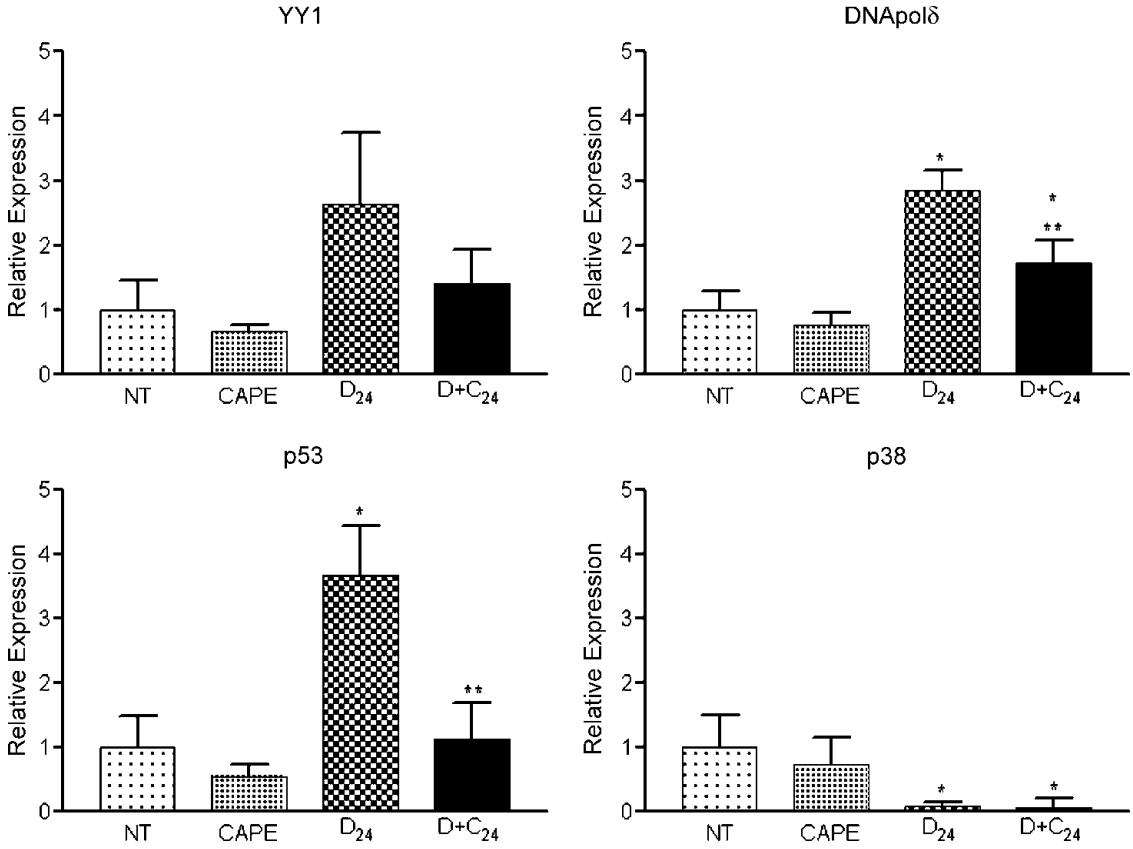


**Figure 5.** RT-PCR validation of *YY1*, *DNA polδ*, *p53*, and *p38* gene expression levels. Samples were analyzed by RT-PCR using primers from Table 1. *CAPE* indicates rats killed 12 hours after CAPE dosing; $D + C_{24}$, killed 24 hours after DEN dosing with CAPE pretreatment 12 hours before DEN ($n = 4$ in each group); $D_{24}$, killed 24 hours after DEN dosing; *NT*, untreated. *$P < .05$ compared with NT, **$P < .05$ compared with $D_{24}$ 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 treat ment, 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 α-2μ-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, *Schizosaccharomyces pombe*, 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 (osteoadherin) (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) (cholecalcin). [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 Bioplot (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 $Na^+$ 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 $K_V6.3$) ($K_V10.1$). [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 *c* oxidase polypeptide VIIA-liver/heart, mitochondrial precursor (EC 1.9.3.1) (cytochrome *c* 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-*trans*-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 *S*-transferase, mitochondrial (EC 2.5.1.18) (GST 13-13) (glutathione *S*-transferase subunit 13) (GST class-kappa). [Source: SWISSPROT; Acc: P24473] | 1.530 |

This list was selected with Bioplot (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 | β 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 | Hepcidin 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 $K^+$ 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 | Fuctinin 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 TERF. [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 α-subunit precursor (EC 3.4.24.18) (endopeptidase-2) (MEP-1) (endopeptidase-24.18 α-subunit) (E-24.18). [Source: SWISSPROT; Acc: Q64230] | 0.654 |
| 9 | G protein–coupled receptor 64. [Source: RefSeq; Acc: NM_181366] | 0.592 |
| 10 | α-2U globulin PGCL4. [Source: RefSeq; Acc: NM_147215] | 0.443 |
| 11 | Dihydropyridine-sensitive L-type, calcium channel β-1 subunit (CAB1) (voltage-dependent calcium channel β-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) (α-2U-globulin) (15.5 kDA fatty acid binding protein) (15.5 kDA FABP) (alpha(2)-euglobulin) (allergen RAT N 1) (RAT N I). [Source: SWISSPROT; Acc: P02761] | 0.514 |
| 15 | Mammary cancer–associated protein RMT-1. [Source: RefSeq; Acc: NM_145088] | 0.539 |
| 16 | α-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) (DHHB methyltransferase) (DHHB-MT) (DHHB-MTASE). [Source: SWISSPRO | 0.658 |
| 18 | Lysophospholipase 1. [Source: RefSeq; Acc: NM_013006] | 0.620 |
| 19 | α-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) (TIP-associated 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) (CYPIIIA1) (P450-PCN1). [Source: SWISSPROT; Acc: P04800] | 0.527 |
| 32 | Testis-specific transporter TST-2. [Source: RefSeq; Acc: NM_173338] | 0.665 |
| 33 | α 2U-globulin (fragment). [Source: SPTREMBL; Acc: Q63220] | 0.522 |
| 34 | Ubiqutin 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) (β-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 | Cortexin. [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–α-trypsin inhibitor heavy chain H3 precursor (ITI heavy chain H3) (inter–α-inhibitor heavy chain 3). [Source: SWISSPROT; Acc: Q63416] | 0.579 |
| 52 | Sodium/potassium–transporting ATPase α-4 chain (EC 3.6.3.9) (sodium pump 4) ($Na^+/K^+$ 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-I) (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) (CYPIIIA18) (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) (CYPIIC13) (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 | α-2-Macroglobulin precursor (α-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-α-L-fucosyltransferase 2 (EC 2.4.1.69) (secretor blood group α-2-fucosyltransferase) (GDP-L-fucose:β-D-galactoside 2-alpha-L-fucosyltransferase 2) (α(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 p38α) (MAP kinase p38α). [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, β-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 (*cis*-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: Q8VII0] | 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 | β-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 α 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 α 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 | α-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 α-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 | Phosphoinositol 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) (kalirin) (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), α subunit (adenylate cyclase-stimulating G α 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 | α-1D Adrenergic receptor (α 1d-adrenoceptor) (α-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: Q8VI99] | 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) (NFI-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-α-hydroxylase) (P450-UT-4). [Source: SWISSPROT; Acc: P15149] | 0.485 |
| 142 | Neuronal acetylcholine receptor protein, α-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 | α-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, α-catalytic subunit (EC 2.7.1.37) (PKA C-α). [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) (HFH-BF-3) (fragment). [Source: SWISSPROT; Acc: Q63246] | 0.580 |
| 167 | α-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, α 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 α-1A subunit (calcium channel, L type, α-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) (CYPIIB1) (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.