Biochemical and Biophysical Research Communications 392 (2010) 473–478

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Emodin inhibits the growth of hepatoma cells: Finding the common anti-cancer pathway using Huh7, Hep3B, and HepG2 cells

Chin-Mu Hsu [a,b], Yu-An Hsu [c], Yuhsin Tsai [a], Fa-Kuen Shieh [d], Su-Hua Huang [e], Lei Wan [a,b,e,*], Fuu-Jen Tsai [b,f,*]

[a] School of Chinese Medicine, China Medical University, Taichung, Taiwan
[b] Genetic Center, China Medical University Hospital, Taichung, Taiwan
[c] Institute of Molecular Medicine, National Tsing Hua University, Hsinchu, Taiwan
[d] Department of Chemistry, National Central University, Jhong-Li, Taiwan
[e] Department of Biotechnology, Asia University, Taichung, Taiwan
[f] School of Post-Baccalaureate Chinese Medicine, College of Chinese Medicine, China Medical University, Taichung, Taiwan

ARTICLE INFO

Article history:
Received 22 October 2009
Available online 4 November 2009

Keywords:
Emodin
G2/M arrest
Hepatocellular carcinoma
Anti-cancer drug

ABSTRACT

Emodin—a major component of *Rheum palmatum* L.—exerts antiproliferative effects in cancer cells that are regulated by different signaling pathways. Hepatocellular carcinoma has high-incidence rates and is associated with poor prognosis and high mortality rates. This study was designed to evaluate the effects of emodin on human hepatocarcinoma cell viability and investigate its mechanisms of action in Huh7, Hep3B, and HepG2 cells. To define the molecular changes associated with this process, expression profiles were compared in emodin-treated hepatoma cells by cDNA microarray hybridization, quantitative RT-PCRs, and Western blot analysis. G2/M phase arrest was observed in all 3 cell lines. Cell cycle regulatory gene analysis showed increased protein levels of cyclin A, cyclin B, Chk2, Cdk2, and P27 in hepatoma cells after time courses of emodin treatment, and Western blot analysis showed decreased protein levels of Cdc25c and P21. Microarray expression profile data and quantitative PCR revealed that 15 representative genes were associated with emodin treatment response in hepatoma cell lines. The RNA expression levels of CYP1A1, CYP1B1, GDF15, SERPINE1, SOS1, RASD1, and MRAS were upregulated and those of NR1H4, PALMD, and TXNIP were downregulated in all three hepatoma cells. Moreover, at 6 h after emodin treatment, the levels of GDF15, CYP1A1, CYP1B1, and CYR61 were upregulated. Here, we show that emodin treatment caused G2/M arrest in liver cancer cells and increased the expression levels of various genes both in mRNA and protein level. It is likely that these genes act as biomarkers for hepatocellular carcinoma therapy.

Introduction

Emodin (1,3,8-trihydroxy-6-methylanthraquinone) is an active component found in the root and rhizome of *Rheum palmatum* L. (Polygonaceae). Emodin is reported to possess antiviral, antiinflammatory, antiulcerogenic, immunosuppressive, and chemopreventive activities. Moreover, antiproliferative effects of emodin have been reported in many cancer cell lines, including cell lines of HER2/neu-overexpressing breast cancer [1], lung cancer [2], leukemia [3], hepatocellular carcinoma [4], cervical cancer, prostate cancer multiple myeloma [5], and neuroblastoma [6]; emodin exerts these effects through activation of caspase-3 [3] and upregulation of TP-53 and p21 [4]. Moreover, emodin inhibits the kinase activity of p56lck, HER2/neu [1], casein kinase II [7], Janus-activated kinase II [5] and the matrix metalloproteinases pathway [6]. However, the molecular mechanisms of emodin-mediated tumor regression have not been completely elucidated thus far.

HCC is the fifth most common and the third most deadly cancer [8], and the most severe complication of chronic liver disease in the world. The annual number of new cases worldwide is approximately 550,000, representing more than 5% of human cancers, and HCC is the third leading cause of cancer-related death [9]. The incidence rates of HCC vary across geographical areas, with high-incidence rates in Eastern Asian and African regions [10]. The incidence is, however, affected by the risk factors of viral hepatitis and dietary aflatoxin exposure, which is increasing in countries with low-incidence rates and even in countries of some high-incidence rates.

Three perpetual cell lines—Huh7, Hep3B, and HepG2—with well-differentiated, epithelial-like cell morphologies were treated with emodin. Huh7 cells were derived from a 53-year-old Japanese man and the Hep3B cells were derived from an 8-year-old black

* Corresponding authors. Address: Genetic Center, China Medical University Hospital, Taichung 404, Taiwan. Fax: +886 4 22033295.
E-mail address: leiwan@mail.cmu.edu.tw (L. Wan).

0006-291X/$ - see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2009.10.153

Table 1
IC_{50} values for inhibition of human hepatoma cell viability by emodin.

	IC_{50} (μM)		
	Huh7	Hep3B	HepG2
Emodin	101.5	66.9	74.36

Inhibiting the proliferation of Huh7, Hep3B, and HepG2 hepatoma cell lines was measured by MTS assay following 72 h of treatment with emodin. Data represent IC_{50} mean values.

man; these 2 cell lines were found to have HBs antigen in the culture supernatant. The HepG2 cells were derived from a Caucasian man and did not have HBs antigen. By using cells with different cell origins, we were able to focus on inhibition of the proliferation of human hepatoma cells by emodin and also examine possible common pathways among the three cell lines.

Materials and methods

Cell culture. Human hepatoma cell lines—Huh7, Hep3B, and HepG2—were obtained from the Bioresource Collection and Research Center (BCRC, Taiwan) and cultured in DMEM with 10% fetal bovine serum (FBS), 2-mM L-glutamine(Biological Industries, Israel), and 10 mg/ml antibiotics (penicillin, and streptomycin; PS; GIBCO). The cell lines were supplied with fresh medium every 3–4 days. Emodin was purchased from Sigma Chemical (St. Louis, MO) and was dissolved in DMSO and maintained as a light-protected 20-mM stock. Emodin was added to the media to a final concentration of 50 μM. Treated and untreated control cells were grown at 37 °C in a humidified atmosphere containing 5% CO_2.

Cell viability assay. Cell viability was measured after 72 h of emodin treatment and assessed using a MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium) cell proliferation assay kit (Promega, USA), in accordance with the manufacturer's instructions. Cell viability was also evaluated by counting cells that excluded trypan blue. All experiments were done at least three times. Data are shown as IC_{50} mean values (Table 1).

Flow cytometry. Cells were treated with emodin (50 and 0 μM) for 24 h and diluted to the concentration of 5×10^7 cells/ml by using staining buffer and a BD Cell Viability Kit (Becton Dickinson, San Jose, CA). Stained cells were immediately analyzed using a BD FACSCanto flow cytometry system (Becton Dickinson, San Jose, CA).

Gene expression profiling by cDNA microarray. To examine the effects of emodin treatment on gene expression in hepatoma cells, Huh7, Hep3B, and HepG2 cells were treated with emodin (50 μM) for 24 h. At the termination of an experiment, total RNA was extracted by using an RNeasy Mini Kit (Qiagen, Valencia, CA) and processed for microarray analysis, as described previously [11]. Affymetrix HG-U133 Plus 2.0 arrays were used as the microarray platform. Arrays were scanned and processed with the Gene-

Table 2
Genes with the greatest upregulation or downregulation following emodin treatment.

Unigene	Gene symbol	Gene name	Fold change	Function
Hs.155569	Chac1	ChaC, cation transport regulator-like 1 (*E. coli*)	3.42	Cation transporter
Hs.154654	Cyp1B1	Cytochrome P450, family 1, subfamily B, polypeptide 1	2.84	Oxygenase
Hs.72912	Cyp1A1	Cytochrome P450, family 1, subfamily A, polypeptide 1	2.68	Oxygenase
Hs.12813	Tiparp	TCDD-inducible poly (ADP-ribose) polymerase	2.23	Nucleic acid binding
Hs.616962	Gdf15	Growth differentiation factor 15	2.08	Growth factor
Hs.414795	Serpine1	Serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1) member 1	1.56	Serine protease inhibitor
Hs.709893	Sos1	Son of sevenless homolog 1 (Drosophila)	1.74	Guanyl-nucleotide exchange factor
Hs.25829	Rasd1	RAS, dexamethasone-induced 1	1.74	Small GTPase
Hs.390594	Slc7A11	Solute carrier family 7 (cationic amino acid transporter, y + system) member 11	1.61	Amino acid transporter
Hs.8867	Cyr61	Cysteine-rich, angiogenic inducer, 61	1.53	Growth factor
Hs.527021	Mras	Muscle RAS oncogene homolog	1.51	Signal transduction; Synaptictransmission
Hs.282735	Nr1H4	Nuclear receptor subfamily 1, group H, member 4	−1.6	Nuclear hormone receptor;Transcription factor;Nucleic acid binding
Hs.483993	Palmd	Palmdelphin	−1.71	Other miscellaneous function protein
Hs.450230	Igfbp3	Insulin-like growth factor binding protein 3	−1.77	Other miscellaneous function protein
Hs.715525	Txnip	Thioredoxin interacting protein	−4.44	Molecular function unclassified

The 15 genes with the greatest increase or decrease in expression following emodin treatment are listed ($P < 0.0001$). All fold-changes are relative to controls.

Table 3
Real-time RT-PCR primer sequences.

Gene	Forward primer	Reverse primer	Annealing temperature (°C)
CHAC1	GGCCCTGAAGTACCTGAATG	GACCTCCTTGGTATCGTAGCC	59
CYP1B1	GCAGCTACACATTTCTCAATCTAAA	CACCAAGGCTGAGACAGTGA	59
CYP1A1	CCCAGCTCAGCTCAGTACCT	GGAGATTGGGAAAAGCATGA	59
TIPARP	CCCTCAAGACTGCCTTTAACC	CTTTCATTATCATCCATTCCAATTT	59
GDF15	CCGGATACTCACGCCAGA	AGAGATACGCAGGTGCAGGT	59
SERPINE1	AAGGCACCTCTGAGAACTTCA	CCCAGGACTAGGCAGGTG	59
SOS1	TCCACGAAGACGACCAGAAT	GGGGACTGTCCAAATGCTTA	59
RASD1	GGTCTACCAGCTCGACATCC	GAACACCAGGATGAAAACGTC	59
SLC7A11	CCATGAACGGTGGTGTGTT	GACCCTCTCGAGACGCAAC	60
CYR61	AAGAAACCCGGATTTGTGAG	GCTGCATTTCTTGCCCTTT	59
MRAS	CAGCTTTGAGCACGTGGA	CGAGGATCATCGGGAATG	59
NR1H4	GAGGAAGACTCAGTCCAGAATCC	CCTTCTACGATGTCTTCTACCTCCT	60
PALMD	TGAGGATCCATCCTTAACAGC	GGTGGTACAACTCTTAGATCACCTT	59
IGFBP3	CCCAGGCTACACCACCAA	GCATATTTGAGCTCCACATTAACTT	59
TXNIP	AAGCTCAAAGCCGAACTTGT	ACGCTTCTTCTGGAAGACCA	59

Chip Scanner 3000 7G and GeneChip operating software (Affymetrix, Santa Clara, CA).

Reverse transcription-PCR. The mRNA expression levels of Huh7, Hep3B, and HepG2 were evaluated using a reverse transcription-PCR (RT-PCR) method and the total cellular RNA was purified using an RNeasy Mini Kit (Qiagen, Valencia, CA). Four micrograms of RNA were reverse-transcribed using the Superscript First Strand synthesis system for conversion to cDNA (Invitrogen, Carlsbad, CA). Primers and probes for amplification and detection were selected from Universal Probes Library (Roche, UK) (Table 3). Amplification was performed in LC480 (Roche, UK) beginning with an initial heating at 95 °C for 10 min followed by 40 cycles of 95 °C for 15 s, 60 °C for 10 s, and 72 °C for 1 s. Levels of gene expression were calculated by computed tomography using glyceraldehydes 3-phosphate dehydrogenase as a control to measure RNA sample integrity.

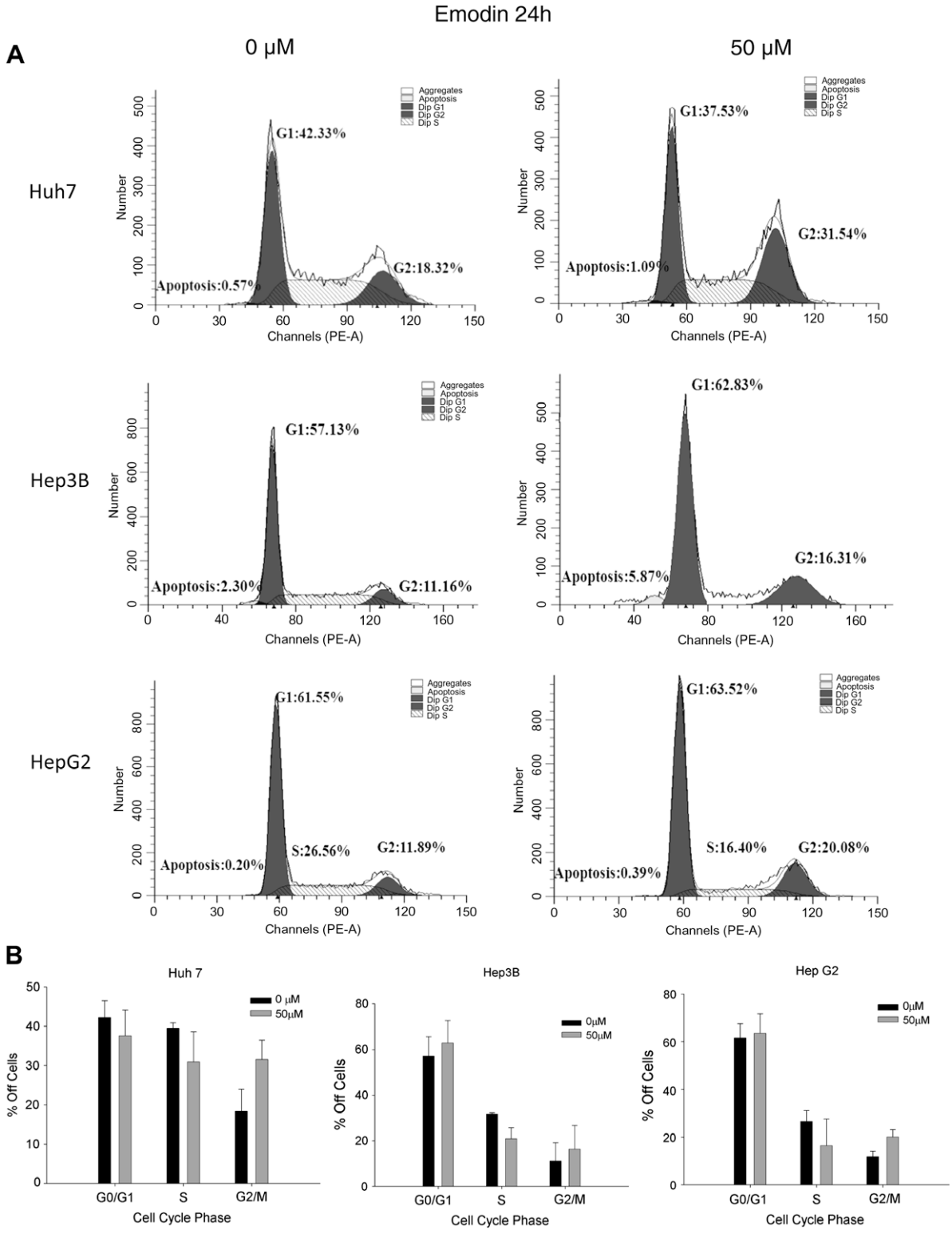

Fig. 1. Treatment of hepatoma cells with emodin resulted in considerable G2/M arrest. Representative flow cytometric graphs of cells treated with emodin (50 μM) for 24 h.

Western blot analysis. Cells were treated with either ethanol (control) or emodin (50 μM) for 0, 6, 12, and 24 h. After treatment, total cell lysates were prepared and 30 μg protein was subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE), followed by immunoblot analysis. Primary antibodies used included anti-cdc25c, cyclin A, cyclin B, chk2, cdk1c, and cdk2 (Cell Signaling, Beverly, MA); p21, p27, cyp1A1 and cyp1B1 (Santa Cruz Biotechnology, Santa Cruz, CA); and cyr61 and gdf15 (R&D Antibodies). Anti-rabbit or anti-mouse secondary antibody conjugated with horseradish peroxidase was also used (Pierce Chromatography Cartridges, USA). Immunoreactive bands were detected by enhanced chemiluminescence (ECL) kit for Western blotting detection by using a ChemiGenius bioimaging system (SYNGENE, USA). Equal loading was confirmed via probing the blots with anti-β-actin antibody (Santa Cruz Biotechnology, Santa Cruz, CA).

Results

Emodin inhibits the growth of hepatoma cells

Emodin is an anthraquinone derivative. The effect of emodin on the growth of hepatoma cells were detected at the concentration of 1, 10, 20, 50, 100, and 150 μM by 72 h by using the MTS assay (Table 1). Cellular growth was inhibited dose-dependently and IC_{50} values observed at 72 h were as follows: 66.9 μM, Hep3B cells; 74.36 μM, HepG2 cells; and 101.5 μM, Huh7 cells. After incubation, we incubated hepatoma cells with 50 μM emodin for 24 h and collected those samples to detect expression levels of genes that were responsible for the growth suppression.

Emodin inhibits cellular growth in the G2/M phase and promotes apoptosis in hepatoma cell lines

Emodin suppressed the proliferation of hepatoma cells after 24 h treatment, arrested cells at the G2/M phase (Fig. 1A), and induced cell apoptosis. The cell numbers increased in apoptosis and at the G2/M phase and decreased at the S phase. The Huh7 cells had the highest percentage difference in number of cells in the G2/M phase, which was 13.22% between the control and 50 μM emodin-treated cells (Fig. 1B).

Emodin-induced changes in hepatoma cell G2/M regulatory proteins

To elucidate the mechanism of emodin-induced growth suppression of hepatoma cells, we next investigated whether the cell-cycle arrest effects of emodin were G2/M phase-dependent. After HepG2 cells were treated with emodin for 0, 6, 12 and 24 h, whole-cell extracts were prepared and analyzed for activation of Cdc25c, cyclin A, cyclin B, Chk2, Cdk1(cdc2), Cdk2, P27 and P21 by Western blotting with β-actin as the control. In response to 50 μM emodin from 0 to 24 h, expression levels of cyclin B, Chk2, Cdk2, and P27 increased whereas emodin-induced expression of Cdc25c and P21 decreased. At 24 h, emodin also reduced cyclin A and Cdk1 expression levels (Fig. 2).

Gene expression profiling changes in response to emodin-induced apoptosis

Emodin-induced gene expression profiling of Huh7, Hep3B, and HepG2 hepatoma cancer cells was investigated by using the GeneChip® Human Genome U133 Plus 2.0 Array (Affymetrix). Cells were treated with and without emodin, and total RNA was obtained after 24 h for microarray analyses. Genes regulated by emodin were determined with a 1.5-fold change cut-off value ($P < 0.0001$). A total of 11 genes were upregulated and 4 genes were downregulated by emodin at 24 h (Table 2). Emodin induced

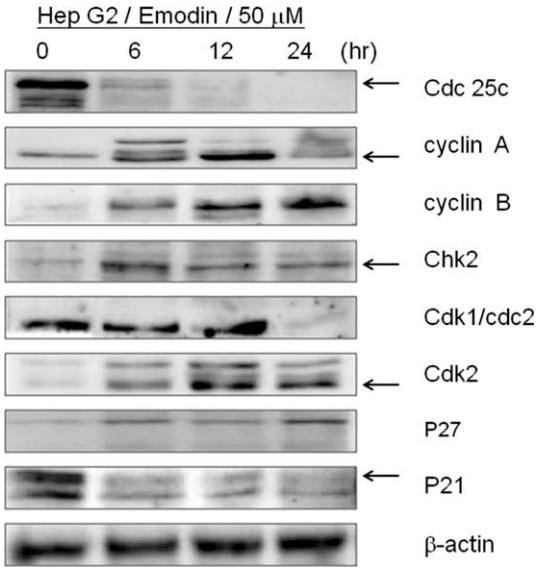

Fig. 2. Emodin-induced changes in G2/M regulatory proteins in HepG2 cells. Cells were treated with either ethanol (control) or emodin (50 μM) for 0, 6, 12, or 24 h. After treatment, total cell lysates were prepared and 30-μg protein was subjected to SDS–PAGE, followed by immunoblot analysis. The expression levels of Cdc25c, cyclin A, cyclin B, Chk2, Cdc2, P27, and P21 were measured and equal loading was confirmed by measurement of β-actin levels. Results are representative of three independent experiments.

the expression of CHAC1, CYP1B1, CYP1A1, TIPARP, GDF15, SERPINE1, SOS1, RASD1, SLC7A11, CYR61, and MRAS, and downregulated the expression of NR1H4, PALMD, IGFBP3, and TXNIP (Table 2).

Gene expression modulation by emodin

To validate the expression level of those genes influence by emodin treatment, quantitative real-time PCRs were performed. All 14 genes that were upregulated or downregulated more than 1.5-fold were chosen as molecular targets of emodin with the GAP-

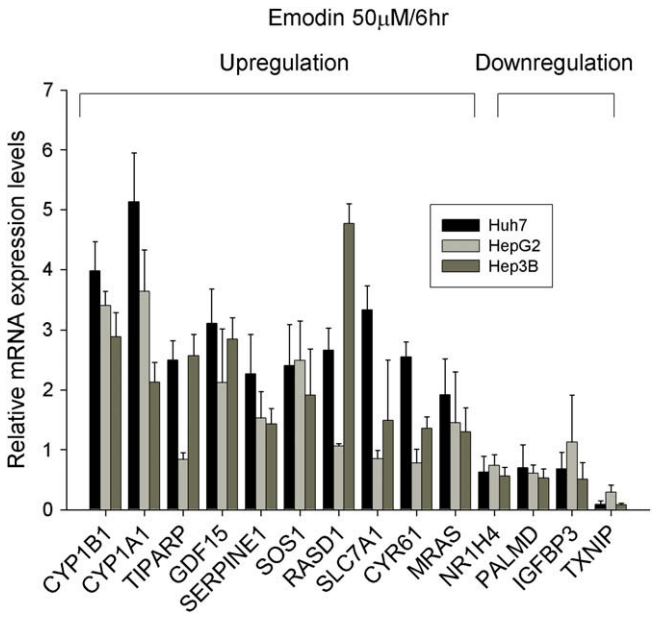

Fig. 3. Gene expression modulated by emodin were detected by real-time PCR using the TaqMan probe and GAPDH as a internal control.

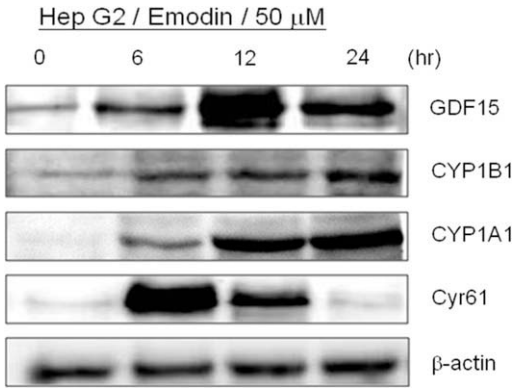

Fig. 4. Measurement of hepatoma cell protein levels after emodin treatment. Proteins were extracted and analyzed by Western blot using β-actin as a loading control.

DH gene as an internal control. Primers and probes used are listed in Table 3. Results of these experiments are summarized in Fig. 3. By using real-time PCR, we confirmed that CYP1A1, CYP1B1, GDF15, MRAS, RASD1, SERPINE1, and SOS1 were upregulated and that NR1H4, PALMD, and TXNIP were downregulated by emodin in the 3 hepatoma cell lines; CYR61, IGFBP3, SLC7A1, and TIPARP were up/downregulated after 6 h of incubation. Furthermore, the mRNA expression levels of all 14 genes were comparable between cDNA microarray and real-time quantitative RT-PCR (Fig. 3).

Measurement of protein levels after emodin treatment

To further confirm these results, HepG2 cells cultured with 50 μM emodin for 0, 6, 12, and 24 h were collected and lysed to detect protein expression by Western blot analysis. As shown in Fig. 4 and 4 genes were selected for detection and β-actin was used as the internal control. Protein expression in the HepG2 cells increased strikingly in a time-dependent manner, similar to the results of microarray and real-time PCR analysis. Protein expression levels of CYP1A1 and CYP1B1 increased with time; the expression of GDF15 was the highest at 12 h and the expression levels of Cyr61 were higher at 6 h than at other times of emodin treatment.

Discussion

Emodin inhibited cell viability of 3 different human hepatoma cell lines—Huh7, Hep3B, and HepG2—indicating that emodin has a broad spectrum of activity against human hepatoma cells. This is the first study to combine cDNA microarray profiling data of 3 cell lines to reveal the anti-tumor pathway of emodin.

Cell-cycle analysis revealed that inhibition of cell viability by emodin was caused by cell-cycle arrest at the G2/M phase, accompanied by a decrease in cell number in the S phase, which indicated apoptotic cell death. These results suggest that cells underwent apoptosis after 24 h of treatment and were manifested as fragmented apoptotic bodies (sub-G0 fraction) after 24 h of treatment. These data emphasize that emodin treatment leads to apoptotic cell death, which is unavoidable. In order to elucidate the mechanism by which emodin induces G2/M arrest, we examined changes in the expression of key proteins that are involved in regulating the cell cycle. The Cdc2/cyclin B complex is the key enzyme regulating the G2 to M transition and is controlled by phosphorylation at various sites [12]. We monitored Cdc2 status at various time points after emodin treatment; at the 24-h time point, a reduction in Cdc2 protein level was documented. In mammalian cells, phosphorylation at Cdc2 inhibitory sites is stabilized in response to DNA damage, delaying mitosis in these cells [13]. After 6 h treatment with emodin, the kinetic behavior of cyclin B was altered and the protein levels increased. Increased levels of cyclin B at early time points may represent the rapid accumulation of cells in the G2 phase when cyclin B is preferentially expressed [14]. In summary, our observations of the changes in Cdc2 and cyclin B expression suggest that emodin-induced G2/M phase arrest in HepG2 cells is mediated by inhibition of Cdc2 activity.

P21waf1, a cyclin-dependent kinase inhibitor (CDKI), binds to and inhibits the activity of the Cdc2/cyclin B complex and causes G2 phase cell-cycle arrest [15]. In order to verify that the observed induction of the Cdc2/cyclin B complex has biological significance in our cellular system, we measured p21waf1 expression levels and found that cells treated with emodin express significantly lower levels of p21waf1 compared to untreated cells. Indeed, increased expression of cyclin B was detected 6 h after emodin treatment. We, therefore, speculate that p21waf1 inactivation likely contributes to cyclin B regulation and the cell-cycle changes observed after emodin treatment.

Human cytochrome P450 (CYP) enzymes play a key role in the metabolism of drugs and environmental chemicals. Oyama et al. showed that the expression levels of CYPs in hepatoma tissues decreased in comparison with those in normal liver tissues [16]. CYP enzymes also could be involved in both activation of pro-anti-cancer drugs and inactivation of anti-cancer drugs. Like tamoxifen, the drug of choice for the treatment of estrogen receptor-positive breast cancer [17], CYPs have been shown to catalyze tamoxifen to 4-hydroxytamoxifen, the activated form of the preventative agent. When cells were treated with emodin for 24 h, CYP1A1 and CYP1B1 were activated as shown in real-time PCR and by time-course expression of proteins. When hepatoma cells were treated with emodin, CYP enzymes were expressed in cancer cells, which could be a relevant clinical factor in tumor sensitivity to these anti-cancer drugs.

Cyr61 (CCN1) is a secreted extracellular matrix associated protein involved in diverse biological functions, including tumorigenesis. When HepG2 cells were treated with 50 μM emodin for 24 h, Cyr61 expression was detected by cDNA microarray and quantitative real-time PCR analysis; in fact, its protein level was increased in most HCC cell lines as detected by Western blot. In a previous study [18], Cyr61 was demonstrated as a tumor suppressor of cell proliferation, adhesion, migration, and invasion in human HCC.

GDF15 (MIC-1) overexpression is associated with different cancers—including gastric, pancreatic, prostate and colorectal—but our data show different results. Perhaps GDF15 in liver cells as previous reports in colon, breast and glioblastoma cell lines has the antitumorigenic role and proceed the induction of apoptosis.

In this study, emodin suppressed cell survival and induced arrest of cultured liver cancer cells at the G2/M phase, which then induced cyclin A, cyclin B, Chk2, Cdk2 and P27 expression and downregulated Cdc25c and P21 expression in time-dependent fashion by Western blot analysis. This is the first report demonstrating that emodin can regulate common gene expression or suppression in different liver cancer cells and the potential biological pathways activated by emodin. Multiple genes—including CYP1A1, CYP1B1, GDF15, SERPINE1, SOS1, RASD1 and MRAS—were upregulated and NR1H4, PALMD, and TXNIP were downregulated at 6 h by real-time PCR, and TIPARP, SLC7A11, and CYR61 gene levels increased but IGFBP3 levels decreased at 24 h detected by microarray technology to identify at hepatoma cell RNA levels the same results as time-dependent protein expression. In conclusion, emodin inhibited hepatoma cell growth and affected the common genes whose regulation is potentially associated with liver tumor progression and could be used as biomarkers to improve hepatocarcinoma diagnosis and treatment.

Acknowledgments

This work was supported by grants from the China Medical University, Taichung, Taiwan (CMU95-133); China Medical University Hospital, Taichung, Taiwan (DMR91-020) and the National Science Council, Taipei, Taiwan (98-2320-B-039-008-MY3).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2009.10.153.

References

[1] S.C. Wang, L. Zhang, G.N. Hortobagyi, M.C. Hung, Targeting HER2: recent developments and future directions for breast cancer patients, Semin. Oncol. 28 (2001) 21–29.
[2] Y.T. Su, H.L. Chang, S.K. Shyue, S.L. Hsu, Emodin induces apoptosis in human lung adenocarcinoma cells through a reactive oxygen species-dependent mitochondrial signaling pathway, Biochem. Pharmacol. 70 (2005) 229–241.
[3] Y.C. Chen, S.C. Shen, W.R. Lee, F.L. Hsu, H.Y. Lin, C.H. Ko, S.W. Tseng, Emodin induces apoptosis in human promyeloleukemic HL-60 cells accompanied by activation of caspase 3 cascade but independent of reactive oxygen species production, Biochem. Pharmacol. 64 (2002) 1713–1724.
[4] D.E. Shieh, Y.Y. Chen, M.H. Yen, L.C. Chiang, C.C. Lin, Emodin-induced apoptosis through p53-dependent pathway in human hepatoma cells, Life Sci. 74 (2004) 2279–2290.
[5] A. Muto, M. Hori, Y. Sasaki, A. Saitoh, I. Yasuda, T. Maekawa, T. Uchida, K. Asakura, T. Nakazato, T. Kaneda, M. Kizaki, Y. Ikeda, T. Yoshida, Emodin has a cytotoxic activity against human multiple myeloma as a Janus-activated kinase 2 inhibitor, Mol. Cancer Ther. 6 (2007) 987–994.
[6] H.F. Lu, K.C. Lai, S.C. Hsu, H.J. Lin, C.L. Kuo, C.L. Liao, J.S. Yang, J.G. Chung, Involvement of matrix metalloproteinases on the inhibition of cells invasion and migration by emodin in human neuroblastoma SH-SY5Y cells, Neurochem. Res. 34 (2009) 1575–1583.
[7] R. Battistutta, S. Sarno, E. De Moliner, E. Papinutto, G. Zanotti, L.A. Pinna, The replacement of ATP by the competitive inhibitor emodin induces conformational modifications in the catalytic site of protein kinase CK2, J. Biol. Chem. 275 (2000) 29618–29622.
[8] D.M. Parkin, F. Bray, J. Ferlay, P. Pisani, Global cancer statistics, 2002, CA Cancer J. Clin. 55 (2005) 74–108.
[9] J.L. Raoul, Natural history of hepatocellular carcinoma and current treatment options, Semin. Nucl. Med. 38 (2008) S13–S18.
[10] P. Pisani, F. Bray, D.M. Parkin, Estimates of the world-wide prevalence of cancer for 25 sites in the adult population, Int. J. Cancer 97 (2002) 72–81.
[11] J. Harbig, R. Sprinkle, S.A. Enkemann, A sequence-based identification of the genes detected by probesets on the Affymetrix U133 plus 2.0 array, Nucleic Acids Res. 33 (2005) e31.
[12] S. Rozenblat, S. Grossman, M. Bergman, H. Gottlieb, Y. Cohen, S. Dovrat, Induction of G2/M arrest and apoptosis by sesquiterpene lactones in human melanoma cell lines, Biochem. Pharmacol. 75 (2008) 369–382.
[13] V.A. Smits, R.H. Medema, Checking out the G(2)/M transition, Biochim. Biophys. Acta 1519 (2001) 1–12.
[14] J. Pines, T. Hunter, Isolation of a human cyclin cDNA: evidence for cyclin mRNA and protein regulation in the cell cycle and for interaction with p34cdc2, Cell 58 (1989) 833–846.
[15] G.K. Schwartz, Development of cell cycle active drugs for the treatment of gastrointestinal cancers: a new approach to cancer therapy, J. Clin. Oncol. 23 (2005) 4499–4508.
[16] T. Oyama, N. Kagawa, N. Kunugita, K. Kitagawa, M. Ogawa, T. Yamaguchi, R. Suzuki, T. Kinaga, Y. Yashima, S. Ozaki, T. Isse, Y.D. Kim, H. Kim, T. Kawamoto, Expression of cytochrome P450 in tumor tissues and its association with cancer development, Front. Biosci. 9 (2004) 1967–1976.
[17] C.K. Osborne, Tamoxifen in the treatment of breast cancer, N. Engl. J. Med. 339 (1998) 1609–1618.
[18] P. Feng, B. Wang, E.C. Ren, Cyr61/CCN1 is a tumor suppressor in human hepatocellular carcinoma and involved in DNA damage response, Int. J. Biochem. Cell Biol. 40 (2008) 98–109.