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

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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

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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