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Microarray analysis of vanillin-regulated gene expression profile in human hepatocarcinoma cells

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Abstract

Vanillin is one of the most widely used flavor compounds in food and personal products. It has been reported that vanillin is able to inhibit mutagenesis induced by chemical and physical mutagens, and to suppress the invasion and migration of cancer cells. Herein we used the oligonucleotide microarray approach to study gene expression profile of vanillin-treated human hepatocarcinoma cells. Microarray data followed by gene ontology (GO) investigation displayed that vanillin-affected clusters of genes involved in cell cycle and apoptosis. Genes down-regulated by vanillin were grouped into three GO categories, regulation of cellular process, cell cycle, and death. Furthermore, most of the down-regulated genes were associated with cancer progression. Knowledge-based analysis further indicated that Fos may play a central role in the regulation of gene expression network. Analysis of Fos-related transcription factor, activator protein 1 (AP-1), showed that vanillin inhibited AP-1 activity in a dose-dependent manner. Furthermore, the phosphorylation of extracellular signal-regulated protein kinase (ERK) was diminished with increasing concentrations of vanillin, indicating that vanillin-regulated AP-1 activity via ERK pathway. In conclusion, our data suggested that vanillin exhibited the anticancer potential by the regulations of cell cycle and apoptosis. Moreover, its regulation may involve the suppression of a central molecule, AP-1.

Keywords: Vanillin; Gene expression profile; Microarray; Activator protein 1; Extracellular signal-regulated protein kinase; Apoptosis; Cell cycle

1. Introduction

Vanillin (4-hydroxy-3-methoxybenzaldehyde) is one of the most widely used flavor compounds in food and personal products, with an estimated annual worldwide consumption of over 2000 tonnes [1]. Vanillin is used extensively in confectioneries, chocolates, butter, ice creams, soft drinks, tobacco, baked foods, puddings, cakes, cookies, and distilled spirits. It is also used as an intermediate in the chemical and pharmaceutical industries for the productions of herbicides, antifoaming agents, and drugs [2]. Vanillin is an important compound with worldwide markets and high commercial values.

Besides its flavor qualities, vanillin possesses the antimicrobial potential and is used as a natural food preservative [3]. Vanillin also exhibits the antioxidant activity by scavenging free radicals and inhibiting the photosensitization-induced lipid peroxidation and protein oxidation [4,5]. Vanillin inhibits the mutagenesis induced by chemical and physical mutagens in both bacteria and mammalian cells [6,7]. It also suppresses UV- and X-ray-induced chromosome aberrations in mammalian cells [8]. Furthermore, it possesses the chemopreventive effect in multi-

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organ carcinogenesis and hepatocarcinogenesis models in rats [9,10]. It also inhibits the invasion and migration of cancer cells, suppresses the matrix metalloproteinase-9 activity, and reduces the numbers of lung metastasized colonies in mice [11].

Vanillin is important in food, chemical, and pharmaceutical industries. Its capacities of antioxidant and anti-mutagen potentiate it to be one of the lead compounds in drug discovery [12]. Here we analyzed the gene expression profile of vanillin in human hepatocarcinoma cells at both the non-cytotoxic and the cytotoxic concentrations by microarray. Gene ontology (GO) and network analysis revealed that genes involved in cell cycle and apoptosis were affected by vanillin. Moreover, most of the genes associated with cancer progression were down-regulated by vanillin. These data suggested that vanillin may exhibit the anticancer potential by the regulations of cell cycle and apoptosis in human hepatocarcinoma cells.

2. Materials and methods

2.1. Oligonucleotide DNA microarrays

Human oligonucleotide DNA microarrays (Human Whole Genome OneArray™) were obtained from Phalanx Biotech Group (Hsinchu, Taiwan). The Human Whole Genome OneArray™ contains 32,050 DNA oligonucleotide probes in a one block array format, and each probe is a 60-mer oligonucleotide designed in the sense direction. Among the probes, 2820 probes correspond to genes annotated in the Biocarta and KEGG databases, 18,824 probes are designed in order to cover all CGAP annotated genes, and 3276 probes are added to complete the Entrez gene probes collection. The probe content is further supplemented by 3783 probes targeting novel transcripts collected in Unigene v175 to make up the total gene probe number to 28,703. Finally, 2265 experimentally defined probes (annotated only by GI number) and 1082 control probes are accounted to make up for the total of 32,050 DNA oligonucleotide probes.

2.2. Cell culture

Human hepatocellular carcinoma cell line (HepG2) was purchased from Bioresource Collection and Research Center (Hsinchu, Taiwan). HepG2 cells secret α-fetoprotein and contain no hepatitis B virus genome [13]. Recombinant HepG2/AP-1 cells, which contain the luciferase gene driven by activator protein 1 (AP-1) responsive elements, were constructed as described previously [14]. Cells were cultured in Dulbecco modified Eagle's medium (DMEM) (Life Technologies, Gaithersburg, MD, USA) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS) (HyClone, Logan, UT, USA), 100 μg/ml streptomycin, and 100 unit/ml penicillin in 75 cm^2 tissue culture flasks in a humidified incubator at 37 °C with 5% CO_2.

2.3. Drug treatment

Vanillin, purchased from Sigma (St. Louis, MO, USA), was dissolved in dimethyl sulfoxide and stocked at −30 °C. Cells were cultured in 25-cm^2 tissue culture flasks before vanillin treatment. Various amounts (0, 1, 5, and 25 mM) of vanillin were added at the time when cells reached a 100% confluence and the vanillin-treated cells were then kept in a humidified incubator at 37 °C with 5% CO_2 for 24 h.

2.4. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay

MTT was purchased from Sigma and dissolved in phosphate-buffered saline (PBS) (137 mM NaCl, 1.4 mM KH_2PO_4, 4.3 mM Na_2HPO_4, 2.7 mM KCl, pH 7.2). Cell viability was monitored by MTT colorimetric assay as described previously [15]. Briefly, cells were cultivated in 96-well culture plates. After a 24-h incubation at 37 °C, various amounts of vanillin were added to confluent cell monolayers and incubated for another 24 h. One-tenth volume of 5 mg/ml MTT was then added to the culture medium. After a 4-h incubation at 37 °C, equal cell culture volume of 0.04N HCl in isopropanol was added to dissolve the MTT formazan, and the absorbance value was measured at 570 nm using a microplate reader. Cell viability (%) was calculated by (OD of vanillin-treated cells/OD of untreated cells). The TC_{50} value was determined as the concentration of vanillin required to inhibit cell viability at 50%.

2.5. Total RNA isolation

Total RNA was extracted from cells treated with or without vanillin using a RNeasy Mini kit (Qiagen, Valencia, CA, USA). Total RNA was quantified using the Beckman DU800 spectrophotometer (Beckman Coulter, Fullerton, CA, USA). Samples with A260/A280 ratios greater than 1.8 were further evaluated using Aglient 2100 bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). The RNA samples with a RNA integrity number greater than 8.0 were accepted for microarray analysis.

2.6. Microarray analysis

Fluorescent cDNA targets were prepared from 20 μg of total RNA samples using Amino Allyl cDNA Labeling Kit (Ambion, Austin, TX, USA) and Cy3/Cy5 dyes (Amersham Pharmacia, Piscataway, NJ, USA). Fluorescent targets were hybridized to the Human Whole Genome OneArray™ with Phalanx hybridization buffer using cover slides. After overnight hybridization at 50 °C, non-specific binding targets were washed away by three different washing steps, and the slides were dried by centrifugation and scanned by an Axon 4000 scanner (Molecular Devices, Sunnyvale, CA, USA). The Cy3 and Cy5 fluorescent intensities of each spot were analyzed by genepix 4.1 software (Molecular Devices). The signal intensity of each spot was corrected by subtracting background signals in the immediate surrounding. We filtered out spots that the flags <0 and intensities < backgrounds or control probes. Spots that passed these criteria were normalized and tested for differential expression by the limma package of the R