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RESEARCH PAPER

Schisandra fructus extract ameliorates doxorubicin-induce cytotoxicity in cardiomyocytes: altered gene expression for detoxification enzymes

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Abstract The effect of *Schisandra fructus* extract (SFE) on doxorubicin (Dox)-induced cardiotoxicity was investigated in H9c2 cardiomyocytes. Dox, which is an antineoplastic drug known to induce cardiomyopathy possibly through production of reactive oxygen species, induced significant cytotoxicity, intracellular reactive oxygen species (ROS), and lipid peroxidation. SFE treatment significantly increased cell survival up to 25%, inhibited intracellular ROS production in a time- and dose-dependent manner, and inhibited lipid peroxidation induced by Dox. In addition, SFE treatment induced expression of cellular glutathione *S*-transferases (GSTs), which function in the detoxification of xenobiotics, and endogenous toxicants including lipid peoxides. Analyses of 31,100 genes using Affymetrix cDNA microarrays showed that SFE treatment up-regulated expression of genes involved in glutathione metabolism and detoxification [GST theta 1, mu 1, and alpha type 2, heme oxygenase 1 (HO-1), and microsomal epoxide hydrolase (mEH)] and energy metabolism [carnitine palmitoyltransferase-1 (CPT-1), transaldolase, and transketolase]. These data indicated that SFE might increase the resistance to cardiac cell injury by Dox, at least partly, together with altering gene expression, especially induction of phase II detoxification enzymes.

Keywords Cardiomyocytes · Cytoprotection · Detoxification · Doxorubicin · Glutathione *S*-transferase · *Schisandra fructus*

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Introduction

Schisandra fructus (SF), the fruit of the *Schisandra chinensis* Baillon, is a well-known traditional herbal medicine that is widely used as a stimulant, a sedative, an antitussive, and a tonic agent in China, Japan, and Korea [9]. It has been reported that SF has various pharmacological activities, including antihepatotoxic [12, 27], anti-inflammatory [14], anticarcinogenic [19], antioxidant, and detoxificant effects [12, 16]. SF contains various active compounds, including essential oils, organic acids, tannins, anthocyanins and lignans [11].

Several in vitro and in vivo studies have shown that SF has potent antioxidative properties such as inhibition of lipid peroxidation, induction of the antioxidant system and scavenging of reactive oxygen species (ROS). Many studies also suggest that SF lignans protect hepatocytes and cortical cells against oxidative damage. These observed cytoprotective effects have been attributed to improvement of the glutathione (GSH) defense system and inhibition of cellular peroxide formation.

Doxorubicin (Dox) is an anthracycline antibiotic that is one of the most effective and widely used anticancer drugs. However, the clinical use of Dox has been limited as its use has been associated with the development of life-threatening cardiomyopathy and congestive heart

failure [4, 20, 23]. Although Dox-induced myocardial dysfunction is multifactorial, the putative main mechanism for Dox-induced cardiotoxicity is the production of free radicals during its intracellular metabolism. Free radicals cause diverse oxidative damage to critical cellular components and membranes in heart tissues [6, 7, 17]. Moreover, the heart is very sensitive to oxidative stress owing to its highly oxidative metabolism, and it has a lower level of antioxidant defense systems than the liver [5].

Considerable efforts have been made to investigate the use of antioxidants to reduce the side effects of Dox administration. In our previous study, we found that anthocyanin, one of the antioxidant components of SF, reduced 5-fluorouracil-induced myelotoxicity [3], Dox-induced cytotoxicity, intracellular ROS production and lipid peroxidation in cardiomyocytes [2]. SF lingans also have been reported to increase Hsp25/70 expression levels and inhibit nuclear factor-κB (NF-κB) activation.

Therefore, we investigated the protective effect of *Shisandra fructus* extract (SFE) on Dox-induced cytotoxicity and its antioxidative properties in H9c2 cardiomyocytes. The SFE-regulated gene expression in the Dox-induced cardiotoxicity system was further characterized using cDNA microarray techniques, which allowed us to systematically understand the cardioprotective mechanisms of SFE at the whole-genome level.

Materials and methods

Extraction of *Schizandra fructus*

Dried fruits of *Schizandra chinensis* were extracted for 3 h with 80% ethanol by using a reflux apparatus to yield the extract after removal of the solvent in vacuo. The ethanol extract was then resolubilized in water and semi-purified by a solid-phase extraction (SPE) with a C18 cartridge (Waters Corp., Milford, MA). The final eluant (SFE) was freeze-dried and resolubilized in phosphate-buffered saline (PBS) for subsequent assays.

Cell culture

H9c2 myocardial cells, spontaneously immortalized ventricular myoblasts of rat embryo, were purchased from the American Type Culture Collection (Manassas, VA). Cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) at 37°C in 5% CO_2. Cell culture medium and supplements were purchased from GibcoBRL (Grand Island, NY). The medium was changed every 2–3 days.

Cell viability

Sub-confluent cells were trypsinized and seeded onto 96-well plates at a density of 1.5×10^5 cells/ml and incubated for 24 h before treatment. Thereafter, cells were exposed to 1 μM Dox for 24 h and then incubated in fresh medium with SFE at a concentration of 30–1,500 μg/ml as a gallic acid equivalent (GAE) for a further 24 h. The effects of SFE on Dox-induced cytotoxicity were assessed using the sulforhodamine B (SRB) assay, as previously described [2, 21]. In brief, after fixation of the cells by the addition of 50% trichloroacetic acid (TCA) solution, the plate was stained with 0.4% SRB solution, and excess dye was then washed out. The unwashed dye was eluted and quantified spectrophotometrically at 550 nm using a microplate reader (Molecular Devices, Sunnyvale, CA). Cell viability was determined as the percentage of surviving cells as compared with that of the Dox-treated control.

Intracellular ROS

Intracellular ROS induced by Dox was measured using 2′7′-dichlorodihydrofluorescein diacetate (H_2DCFDA, Molecular Probes, Eugene, OR) by the method of Wang and Joseph [24] with slight modifications. H9c2 cells were loaded with 20 μM H_2DCFDA for 10 min, followed by two washes with HBSS. The dichlorodihydrofluorescein (DCF) fluorescence was detected using a fluorescence spectrophotometer (BMG LABTECH GmbH, Offenburg, Germany) with the excitation of 485 nm and emission of 520 nm, and fluorescence image was visualized by a fluorescence microscope.

Lipid peroxidation

The thiobarbituric acid reactive substances (TBARS) method was used to determine the effect of SFE on Dox-induced lipid peroxidation according to the method of Jo and Ahn [13] with slight modification. H9c2 cells were washed twice with PBS and lysed in sodium dodecyl sulfate (SDS) solution. The 200 μl of cell lysates were transferred to test tubes and 50 μl of butylated hydroxytoluene (BHT) was added to prevent sporadic lipid peroxidation during heating. Next, a solution of 1.5 ml of 0.5 M HCl, 1.5 ml of 20 mM TBA and distilled water was added. The reaction mixture was well mixed and then heated for 30 min in a boiling water bath. After cooling for 20 min, 2 ml of *n*-butanol was added. The mixture was mixed vigorously and centrifuged at 3,000 rpm for 15 min. The fluorescence of the organic layer was measured through a fluorescence spectrophotometer with an excitation of 515 nm and emission of 555 nm. TBARS levels were calculated from a

standard curve using 1,1,3,3-tetramethoxypropane and normalized to the protein content.

Glutathione *S*-transferase activity

GST activity was assayed spectrophotometrically at 340 nm using the standard substrate 1-chloro-2, 3-dinitrobenzene (CDNB) and co-substrate GSH by the method of Habig et al. [8]. The increase rate of absorbance at 340 nm is directly proportional to GST activity. The activity was calculated using an extinction coefficient of 9.6 mM^{-1} cm^{-1} and expressed as nmol of CDNB–GSH conjugate formed per min per mg protein.

Isolation of total RNA and microarray analysis

The total RNA from the H9c2 cells was isolated using TRIzol (Invitrogen, Carlsbad) and then cleaned using the RNeasy Mini Kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's instructions. Probe synthesis from total RNA (5 μg), hybridization, detection and scanning was performed according to the standard protocols from Affymetrix (Affymetrix Inc., Santa Clara, CA). Briefly, cDNA was synthesized from total RNA using the one-cycle cDNA Synthesis Kit and T7-oligo (dT) primers. Double-stranded cDNA was used for in vitro transcription (IVT). cDNA was transcribed using the GeneChip IVT Labeling Kit (Affymetrix), and 10–15 μg of labeled cRNA was fragmented to form 35–200 bp fragments. After hybridization to the Rat 230 2.0 gene chips (Affymetrix), according to the Affymetrix standard protocol, the arrays were washed and stained with a streptavidin–phycoerythrin complex, and the intensities were determined using a GeneChip scanner 3000 (Affymetrix) and controlled by GCOS Affymetrix software 1.4 (Affymetrix). After data normalization, a DNA microarray data analysis was performed using GenPlex™ (Istech, Korea). The detectable expressed genes were defined using P-, M-, and A-calls according to the Affymetrix algorithm and intensity of the genes. We selected only those genes with at least four P-calls. Cross comparisons of the control versus treated data from each experiment were analyzed on the filtered probes. Only genes that were statistically significant and were up- or down-regulated by at least twofold compared with the control were judged as valid and identified as differentially expressed genes (DEGs).

Statistical analysis

All cytochemical data were given as mean ± SEM. Statistical analysis was carried out using the Student's *t* test. Differences with $p < 0.05$ were accepted as statistically significant. For microarray data, signal intensities between two groups were analyzed by the Welch's *t* test with a significance level of $p < 0.05$.

Results

Modulation of Dox-induced cellular response in H9c2

Treatment of H9c2 cardiomyocytes with Dox and SFE affected cellular responses including cell viability, intracellular ROS production, lipid peroxidation and GST activity.

Dox-induced significant cytotoxicity and the half-maximal inhibitory concentration (IC_{50}) of Dox on the growth of H9c2 cells was determined to be ~1 μM. Thus, this concentration was chosen for all further experiments except the intracellular ROS analysis. Figure 1 shows that SFE has a protective effect against Dox-induced cytotoxicity. The addition of 150 or 300 μg/ml SFE significantly increased cell survival by 15 and 25%, respectively, compared with the Dox-treated control cells ($p < 0.05$).

The effects of Dox and/or SFE on the intracellular ROS formation are shown in Fig. 2. The DCF fluorescence was proportional to the amount of intracellular ROS formed. Cells exposed to Dox showed an increase in DCF fluorescence in a time-dependent manner, whereas in cells

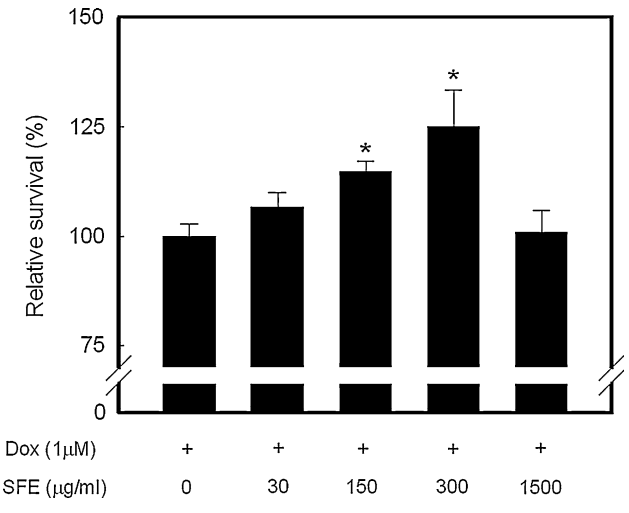

Fig. 1 Cytoprotective activities of SFE on Dox-induced cytotoxicity in H9c2 cardiomyocytes. After 24 h exposure to 1 μM Dox, cells were incubated with SFE for a further 24 h and then assessed for cytotoxicity using the SRB assay. Cell survival is represented by a percentage of cell viability compared with the Dox-treated control. The concentration of SFE was expressed as a gallic acid equivalent (GAE). $^{*}p < 0.05$ compared with the Dox-treated control

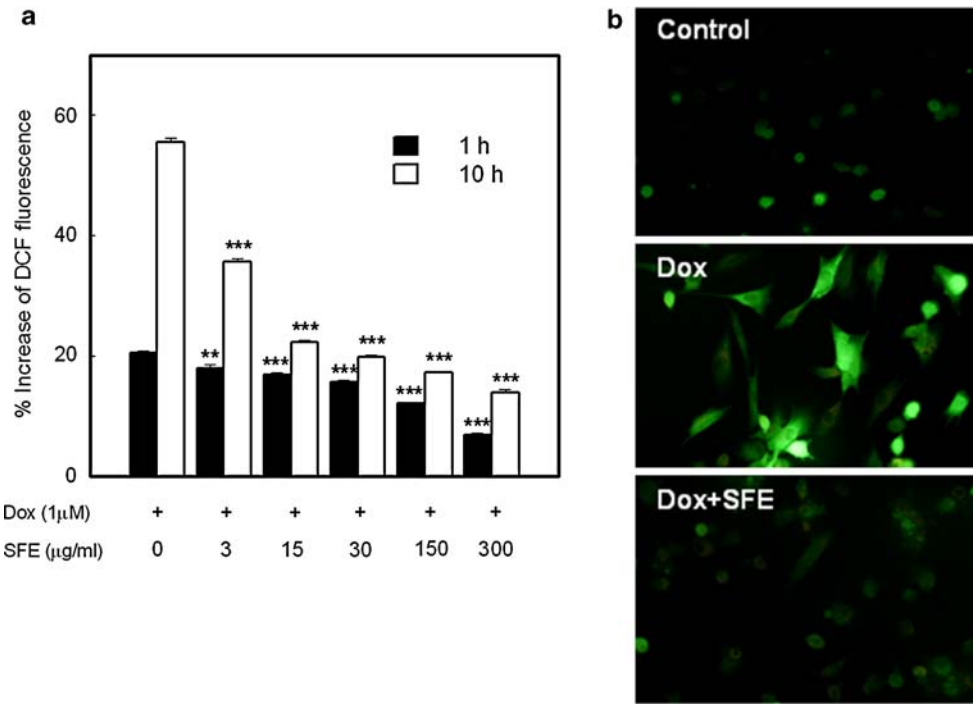

Fig. 2 Scavenging effect of SFE on Dox-induced intracellular ROS formation in H9c2 cardiomyocytes. Production of intracellular ROS was determined by measurement of DCF fluorescence, which was emitted by intracellular oxidation. **a** After 1 and 10 h incubation with 10 μM Dox and SFE, the fluorescence was measured with a fluorescence microplate reader. DCF fluorescence is expressed as % increase in DCF fluorescence with respect to the intensity at 0 time. $^{**}p < 0.01$ and $^{***}p < 0.001$ compared with the Dox-treated control. **b** Fluorescence images show the ROS levels in control cells and the cells stimulated with Dox or Dox and SFE

treated with SFE for 1 h, the DCF fluorescence significantly reduced in a dose-dependent manner, even at the lowest concentration tested (3 μg/ml). After 10 h of incubation time, the percentage increase in DCF fluorescence widened the gap between Dox-treated control and SFE-treated cells.

ROS initiate the autocatalytic chain of lipid peroxidation, resulting in the formation of a variety of toxic species and, ultimately, cell death. Thus, lipid peroxidation has been recognized as a potential mechanism of cell injury. Lipid peroxidation, as measured by the TBARS levels after 24 h exposure of H9c2 cells to 1 μM of Dox, was significantly higher by about 30% compared with the Dox-untreated control ($p < 0.01$). As shown in Fig. 3, treatment with 300 μg/mL SFE treatment significantly reduced the TBARS level to a similar level as that of Dox-untreated control ($p < 0.05$); however, a low concentration of SFE (30 μg/ml) did not have a significant effect on TBARS production.

We also analyzed the activity of cellular GSTs of which the main function is to catalyze the conjugation of electrophilic xenobiotics or their metabolites, including peroxides; the cellular GST activity is shown in Fig. 4, and it can be seen that Dox treatment did not significantly induce GST activity. Treatment with 30 μg/ml SFE increased GST activity significantly by 18 and 24%, respectively, compared with the Dox-treated and untreated controls. However, 300 μg/ml SFE showed a slight increase in GST activity compared with the Dox-treated and untreated controls.

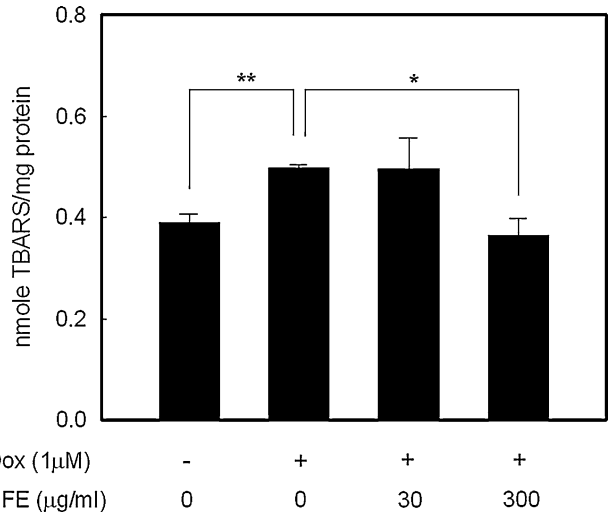

Fig. 3 Effect of SFE treatment on Dox-induced lipid peroxidation in H9c2 cardiomyocytes. After 24 h exposure to 1 μM Dox, cells were incubated with SFE for a further 24 h, and then assessed for lipid peroxidation using the TBARS method. $^{*}p < 0.05$, $^{**}p < 0.01$

Altered gene expression by Dox and SFE treatment in H9c2

The numeric distribution of total genes and the differentially expressed genes (DEG) treated with 1 μM of Dox and/or 300 μg/mL of SFE in H9c2 cardiomyocytes are shown in Fig. 5. Dox and/or SFE treatment in H9c2 cardiomyocytes caused significant changes in gene expression. A total of 568 genes (151 were up-regulated,

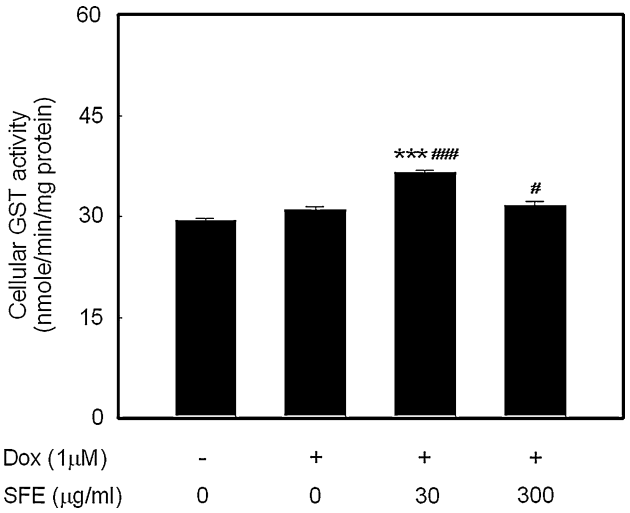

Fig. 4 Effect of SFE treatment on the GST activity in H9c2 cardiomyocytes. After 24 h exposure to 1 μM Dox, cells were incubated with SFE for a further 24 h, and GST activities were then assessed using CDNB. $^{\#}p < 0.05$, $^{\#\#\#}p < 0.001$ compared with untreated control and $^{***}p < 0.001$ compared with Dox-treated control

417 were down-regulated) were differentially expressed with Dox treatment, and 2,015 genes (765 were up-regulated, 1,250 were down-regulated) were differentially expressed with Dox and SFE treatment. After DEGs were selected according to the criteria of a >twofold change in expression and p value <0.05, the DEGs were grouped into nine categories according to the pattern of gene expression: up–up, up–not changed (nc), up–down, nc–up, nc–nc, nc–down, down–up, down–nc, and down–down, based on the responses to Dox treatment, and Dox and SFE treatment. DEGs in the up–up category were up-regulated with Dox treatment compared with control, and up-regulated with Dox and SFE treatment compared with Dox treatment. Those in the up–nc category were up-regulated with Dox treatment compared with control, but showed no change with Dox and SFE treatment compared with Dox treatment. The genes in the up–up category (30), up–down category (0), nc–up category (29), and down–up category (2) were selected as the putative target DEGs involved in the cyto-protection of SFE against Dox-induced cytotoxicity. A total of 61 genes were selected and are listed in Table 1. These target DEGs were grouped into several categories on the basis of their gene ontologies and cellular functions, and included genes involved in glutathione metabolism and detoxification (such as GST theta 1, mu 1, and alpha type 2, and heme oxygenase 1), lipid, carbohydrate and energy metabolism (such as carnitine pamitoyltrasferase 1, trans-aldolase 1, and coenzyme Q6 homolog), protein metabolism, immune response (such as cytokine receptor-like factor 1 and integrin, beta 6), transmembrane transport (such as gap junction membrane channel protein alpha 4), chromosomal protein, apoptosis (such as cycline-dependent kinase inhibitor 1A), cell migration and motility, signal transduction and transcription.

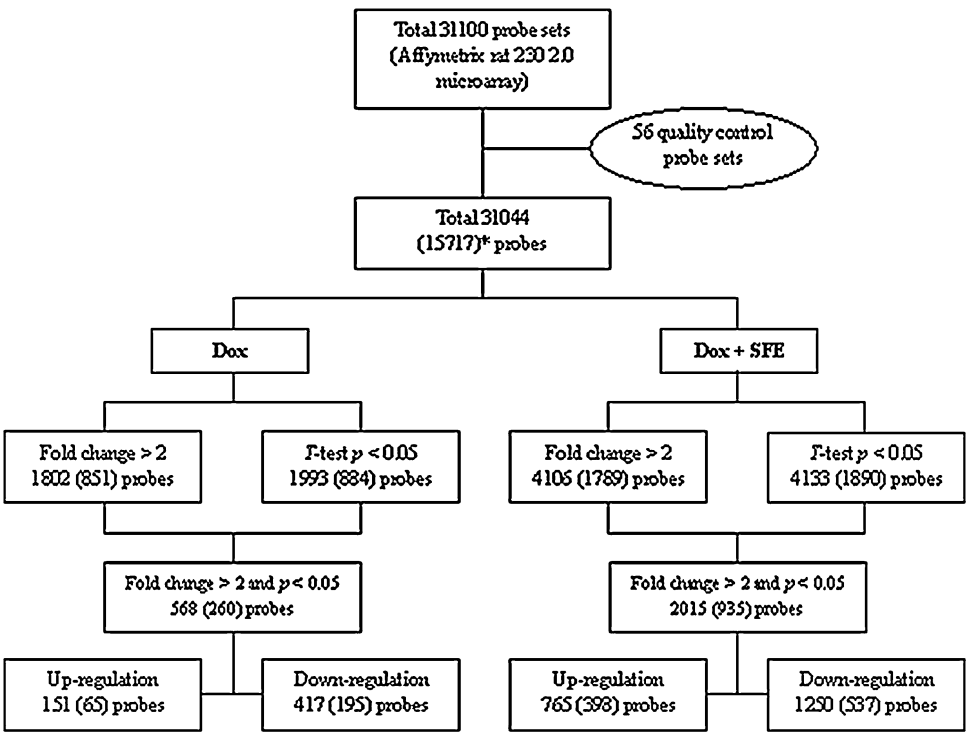

Fig. 5 Diagrammatic representation of the genes tested. The figure shows how the genes were sorted on the basis of statistical criteria applied, as described in the "Methods." **Values in parentheses* denote the number of probes with gene ontology

Table 1 Classified target genes implicated in cytoprotective effects of SFE on Dox-induced toxicity

Accession no.	Description	Expression pattern[a]
Glutathione metabolism and detoxification		
NM_053293	Glutathione *S*-transferase theta 1	up–up
M28241	Glutathione *S*-transferase, mu 1	nc–up
NM_017013	Glutathione *S*-transferase, alpha type2	up–up
NM_053840	Gamma-glutamyltransferase 1	up–up
NM_012580	Heme oxygenase (decycling) 1	up–up
NM_012844	Epoxide hydrolase 1, microsomal	nc–up
Lipid metabolism		
BM390364	Transmembrane 7 superfamily member 2	nc–up
NM_031559	Carnitine palmitoyltransferase 1, liver	up–up
U66322	Leukotriene B4 12-hydroxydehydrogenase	up–up
BI276935	Dehydrogenase/reductase (SDR family) member 3	nc–up
BI293393	Crystallin, lamda 1	nc–up
NM_133586	Carboxylesterase 2 (intestine, liver)	up–up
NM_012715	Adrenomedullin	up–up
AA996933	CGI-58-like protein	up–up
Carbohydrate metabolism		
NM_031510	Isocitrate dehydrogenase 1 (NADP+), soluble	nc–up
NM_134407	Aldo-keto reductase family 7, member A2 (aflatoxin aldehyde reductase)	nc-up
NM_031972	Aldehyde dehydrogenase family 3, member A1	up–up
NM_031811	Transaldolase 1	up–up
NM_022592	Transketolase	up–up
Protein, amino acid metabolism		
J02827	Branched chain ketoacid dehydrogenase E1, alpha polypeptide	nc–up
AI169359	Cathepsin F	up–up
Transmembrane and transport		
NM_021654	gap junction membrane channel protein alpha 4	nc–up
AB043636	Potassium inwardly-rectifying channel, subfamily J, member 8	nc–up
BI285135	Similar to DNA segment, Chr 11, ERTO Doi 18, expressed	nc–up
AI235230	Potassium channel tetramerisation domain containing 13	up–up
AW915385	Golgi associated, gamma adaptin ear containing, ARF binding protein 2	nc–up
NM_133315	Solute carrier family 39 (iron-regulated transporter), member 1	up–up
BM386498	Vacuolar protein sorting 33a (yeast)	nc–up
NM_017154	Xanthine dehydrogenase	nc–up
Immune response		
AA866388	Cytokine receptor-like factor 1 (predicted)	up–up
AF253065	Chemokine-like factor 1	nc–up
AA925583	B-cell linker	nc–up
AI176519	Immediate early response 3	nc–up
NM_030854	Leukocyte cell derived chemotaxin 1	nc–up
AI070686	Integrin, beta 6	down–up
Electron transport, energy metabolism		
AI170570	Coenzyme Q6 homolog (yeast)	up–up
AI179227	Similar to hypothetical protein MGC20446	nc–up
AI501131	Lactate dehydrogenase D	nc–up
Chromosomal protein		
NM_022686	Germinal histone H4 gene	up–up

Table 1 continued

Accession no.	Description	Expression pattern[a]
AI235236	Histone 2, Hh2aa (predicted)	up–up
Apoptosis		
U24174	Cyclin-dependent kinase inhibitor 1A	nc–up
AA818262	Angiopoietin-like protein 4	up–up
Cell migration, cell motility, localization of cell		
AI146115	EPH receptor B1	nc–up
NM_053843	Fc receptor, Igg, low affinity III	up–up
AI112954	*ABI* gene family, member 3	nc–up
Signal transduction		
AB004454	Presenilin 2	up–up
NM_012580	Heme oxygenase (decycling) 1	up–up
Transcription		
BF403357	hpaII tiny fragments locus 9C	nc–up
Others		
AY081218	Pregnancy-induced growth inhibitor	up–up
BF403568	UDP-*N*-acteylglucosamine pyrophosphorylase 1-like 1 (predicted)	up–up
AI059015	Ectonucleoside triphosphate diphosphohydrolase 5	up–up
BI278288	Similar to RIKEN cDNA 0610039C21 (predicted)	up–up
AI235236	Similar to Hist2h2aa1 protein	up–up
BF416240	Hypothetical LOC300751 (predicted)	nc–up
BG375890	Similar to hypothetical protein	nc–up
BI295064	Similar to brain protein 17	nc–up
AI411088	Similar to RIKEN cDNA 4930570c03	up–up
⊥BI296172	Similar to 2310010G13Rik protein	up–up
AI012782	Similar to cell surface receptor FDFACT, similar to paired immunoglobin-like type 2 receptor beta	nc–up
BI294552	Similar to RIKEN cDNA 2310011J03	up–up
AI171553	Similar to RIKEN cDNA 6330406I15 (predicted)	down–up
AI548652	Similar to hypothetical 55.1 kDa protein F09G8.5 in chromosome III	nc–up

[a] Among DEGs grouped into nine categories based on the pattern of gene expression, three categories, up–up, not changed (nc)–up, and down–up, that were considered to be implicated in cytoprotective effects of SFE on Dox-induced toxicity were selected

Discussion

In the present study, Dox treatment significantly increased intracellular ROS production, lipid peroxidation and cytotoxicity in H9c2 cardiomyocytes. The addition of SFE–H9c2 cells provided significant cytoprotection against Dox-induced cytotoxicity and reduced in intracellular ROS and lipid peroxidation.

Aerobic organisms have a multi-tiered defense system to combat oxidative stress, which provides protection not only against ROS, but also against the toxic electrophilic compounds generated by the interaction of ROS with cellular constituents, particularly the lipid peroxidation products. Thus, ROS scavenging is the first line of defense and inactivation of lipid peroxides is the second line of defense. In this study, we found that SFE also directly scavenged the intracellular ROS as the fisrt line of defense and reduced lipid peroxidation as the second line of defense, thereby protecting against Dox-induced cytotoxicity.

The functional classification of target DEGs could provide some clues to the cytoprotective mechanisms of SFE against Dox-induced cytotoxicity. Glutathione metabolism and detoxification-related genes were considered as the primary target genes involved in the cytoprotective action of SFE. GSTs are a large and diverse group of phase II biotransformation enzymes that function in the detoxification of xenobiotics and endogenous toxicants. The compounds detoxified by GSTs include a broad range of carcinogens, anticancer drugs, metabolic byproducts, as well as environmental chemicals. Some GST isozymes, especially alpha class GSTs, can efficiently reduce fatty acid hydroperoxides as well as phospholipid hydroperoxides, and can disrupt the autocatalytic chain of lipid peroxidation by reducing these hydroperoxides that

propagate lipid peroxidation chain reactions [10]. Furthermore, GST alpha 1 and alpha 2 can use membrane phospholipid hydroperoxides as substrates in situ and can protect cell membranes at the sites of damage. The protective role of human GST alpha 2–2 (hGSTA 2–2) against oxidant toxicity has been demonstrated because transfection of K562 cells with hGSTA 2–2 protects these cells from H_2O_2 cytotoxicity [25].

Our microarray results showed that GST theta 1, mu 1, and alpha type 2 genes up-regulated by treatment with Dox and SFE compared with Dox treatment alone, as shown by the levels of cellular GST activity. Recently, it was reported that induction of cellular antioxidants and phase II enzymes, including GST alpha 1, mu 1, and pi 1, by 3H-1, 2-dithiole-3-thione (D3T) provided marked protection against H9c2 cell injury caused by various oxidants and simulated ischemia–reperfusion [1].

In addition, one of the phase II enzymes, heme oxygenase 1 (HO-1), which catalyzes the degradation of heme to release free iron, carbon monoxide and biliverdin, is up-regulated. Some reports have demonstrated the potent antioxidative and cytoprotective properties of heme-derived metabolites generated by HO-1 against various stress stimuli [26]. Microsomal epoxide hydrolase (EHm), which is another phase II enzyme and catalyzes the addition of H_2O across the epoxide to form the corresponding diol [15], was also up-regulated by Dox and SFE treatment. These up-regulated cellular detoxifying systems might increase resistance to cell injury caused by Dox.

Many of the target DEGs that are up-regulated with Dox and SFE treatment are involved in lipid, carbohydrate and energy metabolism. Carnitine palmitoyltransferase-1 (CPT-1), which is an integral outer mitochondrial membrane protein and the key regulatory enzyme of fatty acid oxidation, was up-regulated by SFE; long-chain fatty acids are the major substrates for energy production in the aerobic adult myocardium [18]. Transaldolase and transketolase also were up-regulated with SFE treatment. These enzymes are involved in the pentose phosphate pathway (PPP), which produces NADPH as a reducing equivalent for biosynthetic reactions and ribose 5-phosphate for the synthesis of nucleotides, RNA and DNA. This up-regulation of lipid, carbohydrate and energy metabolism-related genes can be partially helpful to the cytoprotective action of SFE, because the heart has high energy demands for maintenance of cellular processes, and abnormalities in the metabolic pathway that reduce energy production, transfer and utilization is one cause of heart failure [22].

In conclusion, this study demonstrates that SFE reduced Dox-induced cytotoxicity and intracellular ROS and lipid peroxidation, and increased cellular GST activity in H9c2 cardiomyocytes. The cDNA microarray data showed that SFE treatment modulates the gene expression of phase II detoxification enzymes such as GSTs, HO-1 and EHm, indicating that SFE might increase the resistance to cardiac cell injury by Dox via induction of the cellular detoxifying system. Future work will be needed to confirm our array results and to determine the functional role of these genes in the reduction of Dox toxicity.

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