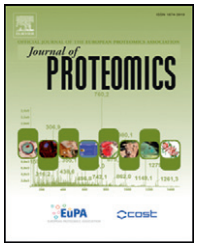
JOURNAL OF PROTEOMICS 75 (2012) 4833–4843

Available online at www.sciencedirect.com

SciVerse ScienceDirect

www.elsevier.com/locate/jprot



Heat shock protein 27 mediates the effect of 1,3,5-trihydroxy-13, 13-dimethyl-2H-pyran [7,6-b] xanthone on mitochondrial apoptosis in hepatocellular carcinoma☆

Wei-ming Fu[a,1], *Jin-fang Zhang*[b,1], *Hua Wang*[a], *Zhi-chao Xi*[c], *Wei-mao Wang*[b], *Peng Zhuang*[d], *Xiao Zhu*[e], *Shih-chi Chen*[f], *Tak-ming Chan*[g], *Kwong-Sak Leung*[g], *Gang Lu*[h], *Hong-Xi Xu*[c,*], *Hsiang-fu Kung*[a,b,**]

[a]*Stanley Ho Centre for Emerging Infectious Diseases, The Chinese University of Hong Kong, Hong Kong, China*
[b]*School of Biomedical Sciences, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong, China*
[c]*Shanghai University of Traditional Chinese Medicine, Shanghai, PR China*
[d]*Department of Infectious Diseases, the Fourth People's Hospital of Shenzhen, 518033, Shenzhen, PR China*
[e]*Key Laboratory of Molecular Diagnosis, Institute of Biochemistry and Molecular Biology, Guangdong Medical College, Dongguan, PR China*
[f]*Department of Mechanical and Automation Engineering, The Chinese University of Hong Kong, Hong Kong, China*
[g]*Department of Computer Science & Engineering, The Chinese University of Hong Kong, Shatin, Hong Kong, China*
[h]*Brain Tumor Center, Neurosurgery, Faculty of Medicine, The Chinese University of Hong Kong, Prince of Wales Hospital, Shatin, Hong Kong, China*

ARTICLE INFO

Article history:
Received 13 March 2012
Accepted 18 May 2012
Available online 5 June 2012

Keywords:
1,3,5-trihydroxy-13,13-dimethyl-2H-pyran [7,6-b] xanthone
Hsp27
Mitochondrial apoptosis
Hepatocellular carcinoma

ABSTRACT

Hepatocellular carcinoma (HCC) is a global public health problem which causes approximately 500,000 deaths annually. Considering that the limited therapeutic options for HCC, novel therapeutic targets and drugs are urgently needed. In this study, we discovered that 1,3,5-trihydroxy-13,13-dimethyl-2H-pyran [7,6-b] xanthone (TDP), isolated from the traditional Chinese medicinal herb, *Garcinia oblongifolia*, effectively inhibited cell growth and induced the caspase-dependent mitochondrial apoptosis in HCC. A two-dimensional gel electrophoresis and mass spectrometry-based comparative proteomics were performed to find the molecular targets of TDP in HCC cells. Eighteen proteins were identified as differently expressed, with Hsp27 protein being one of the most significantly down-regulated proteins induced by TDP. In addition, the following gain- and loss-of-function studies indicated that Hsp27 mediates mitochondrial apoptosis induced by TDP. Furthermore, a nude mice model also demonstrated the suppressive effect of TDP on HCC. Our study suggests that TDP plays apoptosis-inducing roles by strongly suppressing the Hsp27 expression that is specifically associated with the

Abbreviations: TDP, 1,3,5-trihydroxy-13,13-dimethyl-2H-pyran [7,6-b] xanthone; *Garcinia oblongifolia*, *G. oblongifolia*; Hsp27, heat shock protein 27; HCC, hepatocellular carcinoma; 2-DE, 2-dimensional electrophoresis; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; Lv-Hsp27, Lenti-Hsp27 vector; Hsp27-si, Hsp27 small interfering RNA; DTT, dithiothreitol.

☆ Disclosure of potential conflicts: The authors declare no conflict of interests.

* *Correspondence to:* H.-X. Xu, Shanghai University of Traditional Chinese Medicine, PR China.
** *Correspondence to:* H.-F. Kung, School of Biomedical Sciences, The Chinese University of Hong Kong, Hong Kong, China. Tel.: +852 2603 7743; fax: +852 2994 4988.
E-mail addresses: zhangjf06@gmail.com (J. Zhang), xuhongxi88@gmail.com (H-X. Xu), b110473@mailserv.cuhk.edu.hk (H. Kung).

[1] These authors contributed equally to this paper.

1874-3919/$ – see front matter
doi:10.1016/j.jprot.2012.05.032

mitochondrial death of the caspase-dependent pathway. In conclusion, TDP may be a potential anti-cancer drug candidate, especially to cancers with an abnormally high expression of Hsp27.

1. Introduction

Hepatocellular carcinoma (HCC), the most common primary hepatic malignancy, is the third leading cause of cancer-related deaths worldwide; chronic hepatitis B and hepatitis C infections are the major risk factors for the development of HCC. In recent years, the incidence of HCC has been rising in the developing countries and most developed countries [1–3]. Although some significant advances have been made in HCC treatments, poor prognoses and high recurrence risks have been a major challenge to researchers. Currently, surgical resections are the main treatment option for HCC patients; however, the complexities arising from surgeries can reduce the therapeutic effect and the patients' survival rate [4–6]. Accordingly, it is urgent to find more effective and alternative therapeutic strategies which may benefit HCC patients.

G. oblongifolia is a medium-sized shrub found in southern China and northern Vietnam. It has been used for many years as a folk medicine in China to treat burns [7], and recent studies have shown that *G. oblongifolia* has apoptosis-inducing activity against Hela cells [8]. However, the active chemical components and their molecular targets remain unclear.

In the present study, we identified 1,3,5-trihydroxy-13,13-dimethyl-2H-pyran [7,6-b] xanthone (TDP), derived from *G. oblongifolia*, as a potent anti-cancer drug candidate to HCC. Furthermore, we explored the underlying molecular mechanism of this compound in HCC. Finally, we also investigated TDP on HCC tumorigenesis in a murine model. Our data suggest that TDP may be a promising anti-cancer drug candidate.

2. Material and methods

2.1. Preparation of TDP

The TDP, isolated from *G. oblongifolia*, was kindly provided by Professor Hong-Xi Xu (Shanghai University of Traditional Chinese Medicine) and its purity was determined to be over 98% by HPLC. Its chemical structure is shown in Fig. 1A. TDP samples were dissolved in DMSO (Sigma-Aldrich, St. Louis, MO, USA) at a stock concentration of 33 mM before use.

2.2. Cell lines and culture

Hepatocellular carcinoma cell lines including HepG2, Hep3B, Bel7404 and Huh-7 and immortalized normal liver cells MIHA and LO2, were cultured in Dulbecco's modified Eagle's medium (DMEM, GIBCO, Green Island, NY, USA) supplemented with 10% fetal bovine serum (FBS, GIBCO) and 1% penicillin/streptomycin (P/S, GIBCO) at 37 °C with 5% CO_2.

2.3. Clinical specimens

Thirty-five paired primary HCC specimens and matched adjacent non-tumor liver tissues were collected from tumor resections at the Prince of Wales Hospital of The Chinese University of Hong Kong (CUHK). All tissues were confirmed by histological staining. Among them, thirty-one paired specimens were from male patients and four paired specimens were from female patients. At the collection, twenty-nine patients were defined as stage II and six patients were classified between stage II and stage III (TNM classification). Informed consents were obtained from all patients. This study was approved by the Ethics Committee.

2.4. Cell viability and apoptosis assay

5.0×10^3 cells per well were seeded in a 96-well plate and the cells were treated with various concentrations of TDP within 24 h. DMSO (0.1%) was used as control. The effect of TDP on cell growth and viability was determined by an MTT assay as described in [9]. For apoptosis analysis, cells were seeded at a density of 2×10^5 cells per well in a 6-well plate and TDP was introduced at a final concentration of 8 μM for 72 h. The quantification of apoptotic cells was performed by a FITC-labeled AnnexinV/propidium iodide (PI) Apoptosis Detection Kit (Invitrogen, Carlsbad, CA, USA), as described in [9].

2.5. Two-dimensional electrophoresis (2-DE), protein visualization and image analysis

Differently treated HepG2 cells were harvested and 2-DE was performed as described in [10]. In brief, isoelectric focusing (IEF) was performed by using IPG strips (13 cm, pH 3–10). The samples were diluted and loaded into IPG strips to run for 10 h at 30 V with IPGphor II horizontal electrophoresis apparatus (GE Healthcare, Buckinghamshire, UK); the IEF was then performed in accordance with the following manner: 500 V and 1000 V for 1 h and then 8000 V for 6 h. After the completion of IEF, the IPG strips were incubated in an equilibration buffer (6 M urea, 2% SDS, 30% glycerol, 0.002% bromophenol blue, 50 mM Tris–HCl and pH 6.8) supplemented with 1% DTT for 15 min under shaking. After transferring to another equilibration buffer that contains 2.5% iodoacetamide for another 15 min, the strips were loaded into 12.5% uniform polyacrylamide gel slabs ($150\times158\times1.5\ mm^3$) for a second dimensional protein separation which was performed by using a 15 mA constant current for 30 min and thereafter by using a 30 mA current.

The procedures following the 2-DE were described in [11]. In brief, after the 2-DE was completed, gels were visualized by