ARTICLE
Journal of Cellular Biochemistry 112:217–224 (2011)

Journal of Cellular Biochemistry

Furanodienone Inhibits Cell Proliferation and Survival by Suppressing ERα Signaling in Human Breast Cancer MCF-7 Cells

Ying-Wei Li, Guo-Yuan Zhu, Xiao-Ling Shen, Jian-Hong Chu, Zhi-Ling Yu, and Wang-Fun Fong*

Center for Cancer and Inflammation Research, School of Chinese Medicine, Hong Kong Baptist University, Kowloon Tong, Hong Kong, China

ABSTRACT

Estrogen receptor alpha (ERα) plays an important role in the development and progression of breast cancer and thus the attenuation of ERα activities is a promising treatment strategy. Furanodienone is one of the main bioactive chemical components of Rhizoma Curcumae which is commonly used in Chinese medicine for the treatment of cancer. In this study, we investigated the effects of furanodienone on human breast cancer MCF-7, T47D, and MDA-MB-231 cells. Our results showed that furanodienone could inhibit MCF-7, T47D, and MDA-MB-231 cells proliferation in a dose (10–160 μM) dependent manner. ERα-negative MDA-MB-231 cells were less sensitive to furanodienone than ERα-positive MCF-7 and T47D cells. Furanodienone could effectively block 17β-estradiol (E2)-stimulated MCF-7 cell proliferation and cell cycle progression and induce apoptosis evidenced by the flow cytometric detection of sub-G1 DNA content and the appearance of apoptotic nuclei after DAPI staining. Furanodienone specifically down-regulated ERα protein and mRNA expression levels without altering ERβ expression. Furanodienone treatment inhibited E2-stimulation of estrogen response element (ERE)-driven reporter plasmid activity and ablated E2-targeted gene (e.g., c-Myc, Bcl-2, and cyclin D1) expression which resulted in the inhibition of cell cycle progression and cell proliferation, and in the induction of apoptosis. Knockdown of ERα in MCF-7 cells by ERα-specific siRNA decreased the cell growth inhibitory effect of furanodienone. These findings suggest that effects of furanodienone on MCF-7 cells are mediated, at least in part, by inhibiting ERα signaling. J. Cell. Biochem. 112: 217–224, 2011.

KEY WORDS: BREAST CANCER; ESTROGEN RECEPTOR ALPHA; FURANODIENONE; MCF-7

In recent years, multiple-target strategies that concurrently inhibit key molecules and pathways important for tumor growth and progression have attracted significant attention in the field of cancer therapy. Among various attempts, endocrine therapy blocking estrogen receptor alpha (ERα) activity as well as elements of related signal pathways have been shown to be a promising approach in the treatment of ERα positive breast cancer [Morandi, 2005; Ding et al., 2007; Arpino et al., 2008].

Anti-estrogen therapies may have two different but synergistic approaches. One approach designed to interfere with hormone-receptor (estrogen-ERα) binding have been proven effective in reducing estrogen-dependent tumor growth. Alternative to targeting directly at ERα function, strategies designed to lower ERα protein level are also useful in the management of hormone-dependent, ERα-positive tumors. However, not all ERα positive breast cancers respond to chemicals designed to have one or both of the above two functions [Massarweh and Schiff, 2006; Jordan and Brodie, 2007; Srinivasan et al., 2009].

Plants are rich sources of novel bioactive chemicals and could provide, directly or after structural modifications, potential effective new drugs. The dried rhizome of *Curcuma phaeocaulis* Valeton (Rhizoma Curcumae), a commonly used traditional Chinese medicinal herb, is clinically used in China for the treatment of liver cancer, cervix cancer, and leukemia [Ma et al., 2008]. Recently, several compounds isolated from Rhizoma Curcumae have been reported to possess anti-cancer activities [Li et al., 2005; Wang et al., 2005; Xiao et al., 2007; Ma et al., 2008]. Furanodienone (Fig. 1A), one of the main bioactive constituents of Rhizoma Curcumae, has

Additional supporting information may be found in the online version of this article.

Grant sponsor: Faculty Research Grant of Hong Kong Baptist University; Grant number: FRG2/08-09/102.

*Correspondence to: Prof. Wang-Fun Fong, PhD, School of Chinese Medicine, Hong Kong Baptist University, Kowloon Tong, Hong Kong, China. E-mail: wffong@hkbu.edu.hk

Received 13 May 2010; Accepted 6 October 2010 • DOI 10.1002/jcb.22922 • © 2010 Wiley-Liss, Inc.
Published online 10 November 2010 in Wiley Online Library (wileyonlinelibrary.com).

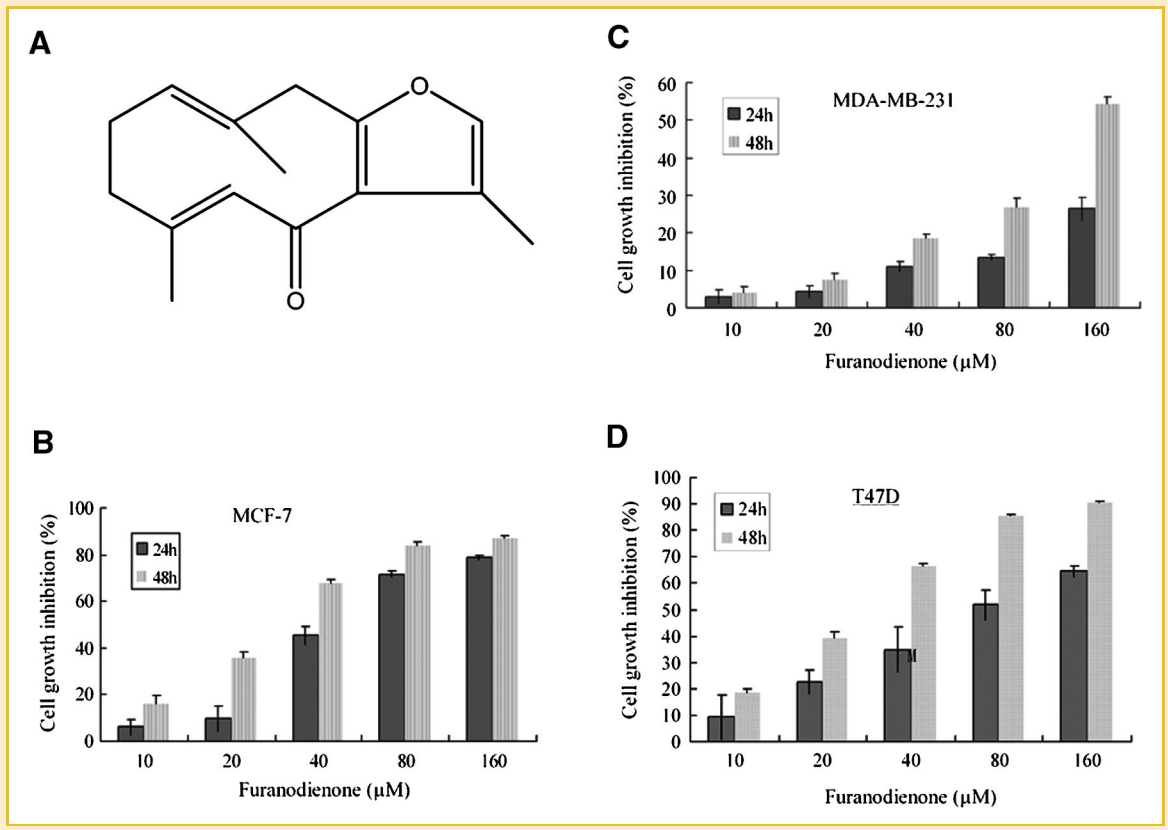

Fig. 1. Chemical structure of furanodienone and effects of furanodienone on MCF-7, T47D, and MDA-MB-231 cells proliferation. A: Chemical structure of furanodienone. (B) MCF-7 cells, (C) MDA-MB-231 cells, and (D) T47D cells were treated with various concentrations of furanodienone (10–160 μM) for indicated durations. Cell growth was assessed by SRB assay. Data shown are means ± SD from three independent determinations.

been reported to have anti-inflammatory activity [Makabe et al., 2006; Tanaka et al., 2008]. Here we report, for the first time, the anti-proliferation effect of furanodienone on ERα-positive MCF-7 human breast cancer cells. Mechanistic studies revealed that the MCF-7 cell growth inhibiting activity was mediated, at least in part, by inhibiting ERα signaling. Our findings suggest that furanodienone, and compounds with related structures, may represent a novel class of chemicals with activities in the prevention and treatment of ERα-dependent human breast cancers.

MATERIALS AND METHODS

REAGENTS AND ANTIBODIES

Furanodienone was isolated in our laboratories from Rhizoma Curcumae. The chemical structure of furanodienone was verified by MS and NMR analyses (Fig. 1A) and the sample used in our studies had a purity >99% as determined by HPLC. Protocols for furanodienone isolation and identification are available upon request. A stock solution of furanodienone was prepared in DMSO and stored at −20°C. E2, Ribonuclease A (RNase A), sulphorhodamine B (SRB), and propidium iodide (PI) were purchased from Sigma–Aldrich (St. Louis, MO). ERα, c-Myc, Bcl-2, cyclin D1, and Gapdh antibodies were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Anti-mouse and anti-rabbit IgG antibodies labeled with horseradish peroxidase were purchased from Amersham Biosciences (Piscataway, NJ). Lipofectamine 2000 was obtained from Invitrogen Corporation (Carlsbad, CA). Bright-Glo luciferase assay system and Beta-Glo assay system were obtained from Promega (Madison, WI). ERE-Luc was kindly provided by Dr. Anfernee Tse (Hong Kong Baptist University, Hong Kong). Control siRNA and ERα siRNA were purchased from Santa Cruz Biotechnology, Inc.

CELL CULTURE AND FURANODIENONE TREATMENT

The human breast cancer cell line MCF-7, T47D, and MDA-MB-231 were obtained from American Type Culture Collection (Manassas, VA). Cells were cultured in DMEM medium (for MCF-7 and MDA-MB-231) or RPMI-1640 medium (for T47D) containing 10% fetal bovine serum, 100 U/ml penicillin, and 100 μg/ml streptomycin (Gibco, New York) at 37°C in a 5% CO_2 humidified atmosphere.

Furanodienone stock solution (dissolved in DMSO) was diluted with culture medium to various concentrations before adding to cultured cells. In all experiments the final concentration of DMSO was <0.05% (v/v).

CELL GROWTH ASSAY

Cells were seeded in 96-well plates at a density of 5×10^3 cells per well and cultured for 24 h. Drugs were added and plates were

incubated for another 24 or 48 h. Cell growth was evaluated by SRB protein staining assay [Houghton et al., 2007] and expressed as percentage of vehicle-treated control [Wang et al., 2007]. To investigate effects of furanodienone on E2-stimulated cell proliferation, MCF-7 cells were grown in phenol red-free medium containing 5% charcoal dextran-stripped fetal bovine serum. Cells were treated with various concentrations of furanodienone with or without E2 for 48 h.

FLOW CYTOMETRIC ANALYSIS OF CELLULAR DNA CONTENT

MCF-7 cells were plated onto six-well tissue culture dishes and grown in phenol red-free medium containing dextran charcoal-stripped 5% fetal bovine serum. Cells were treated with 10 nM E2 in the presence or absence of 20–80 μM furanodienone for 48 h. Cells were collected by centrifugation (500*g* for 5 min at 4°C) and washed twice with PBS. Washed cells were fixed in 70% ethanol and stored at −20°C. Before flow cytometry analysis, cells were washed in PBS and stained by suspension in PI (50 mg/L) containing RNaseA (2 mg/L) for 15 min at 37°C. Stained cells were analyzed using a FACScan flow cytometer (BD Biosciences, USA) [Ricote et al., 2006].

APOPTOSIS DETECTION BY DAPI STAINING

Cells cultured on slides were grown in phenol red-free medium containing dextran charcoal-stripped 5% fetal bovine serum. Cells were treated with 10 nM E2 in the presence or absence of 20–80 μM furanodienone for 48 h. After treatment, cells on slides were washed in PBS and fixed with 4% paraformaldehyde for 10 min. After 10 min incubation with DAPI, cells on slides were examined by fluorescence microscopy (Nikon, Japan).

WESTERN BLOT ANALYSIS

After furanodienone treatment growth medium was removed and cells were washed with ice-cold PBS and lysed in a modified RIPA buffer (in mM: 50 Tris–Cl, 150 NaCl, 1 EDTA, 1 EGTA, 1 phenylmethylsulfonyl fluoride, 1 NaF, 1 Na_3VO_4, and 1% v/v NP-40, 0.35% w/v sodium-deoxycholate, 10 μg/mL each of aprotinin, leupeptin, and pepstatin A, pH adjusted to 7.4) for 20 min at 4°C. After centrifugation at 14,000*g* for 15 min at 4°C, the supernatant was collected and regarded as whole cell extract. Samples containing 30–50 μg of protein were separated by SDS–polyacrylamide gel electrophoresis and then transferred onto nitrocellulose membranes (0.45 μm, Bio-Rad) that were subsequently immunoblotted with primary antibodies followed by horseradish peroxidase-conjugated secondary antibodies (1:3,000). Labeled protein spots were visualized by ECL (Amersham Biosciences) according to the manufacturer's instruction [Tse et al., 2007b]. The density of each band was quantified using the ImageJ 1.41o (NIH, USA).

REVERSE TRANSCRIPTION-PCR (RT-PCR)

MCF-7 cells were harvested in Trizol Reagent (Invitrogen Corporation), and total RNA was extracted following the manufacturer's protocol. One microgram of total RNA was subjected to a RT reaction using random oligonucleotide primers and M-MLV reverse transcriptase (Promega). One microliter of the RT reaction product was then amplified by PCR using HotStar Taq DNA polymerase (Qiagen) at 95°C for 1 min, 55°C for 1 min, and 72°C for 1 min. The primers used for amplification were: ERα, forward AGATCTTCGACATGCTGCTG and reverse CACTTCATGCTGTACAGATG; c-Myc, forward AACCAG AGTTTCATCTGC GACCCG and reverse TTGTGCTGATGTGTGGAGACGTGG; Bcl-2, forward ACAACATCG CCCTGTGGATGAC and reverse ATAGCTGATTCGACGTTTTGCC; cyclin D1, forward CTGGAGAGG AAGCGTGTGAGG and reverse CTGGAGCCCG TGAAAAAGAGC; and actin, forward GATGATATCGCCGCGCTCGTCGTCGAC and reverse AGCCAGGTCCAGACGCAGGATGGCATG. PCR products were separated using a 1.5% agarose gel and identified by ethidium bromide staining [Tse et al., 2007a]. The density of each band was quantified using the ImageJ 1.41o (NIH).

ESTROGEN RESPONSE ELEMENT-LUCIFERASE ASSAY

MCF-7 cells were plated in 24-well plates and transfected with ERE-luciferase and β-gal reporter plasmids using Lipofectamine 2000 (Invitrogen Corporation) according to the manufacturer's protocol. The ratio of the ERE-luciferase plasmid and the β-gal plasmid is 1:1. The DNA/lipofectamine mixture was removed 4 h later, and cells were treated with 10 nM E2 and various concentrations of furanodienone in phenol red-free medium containing dextran charcoal-stripped 5% fetal bovine serum. Cell extracts were obtained after 24 h and luciferase activity was measured by a Dual Luciferase Reporter Assay System (Promega). Cells were lysed with 100 μL of 1× reporter lysis buffer, and 30 μL of cell extract were used for luciferase and β-galactosidase assays. Luciferase activities were normalized with β-galactosidase activity. The luciferase activity in the MCF-7 cells treated for 24 h with 10 nM E2 alone was taken as the 100% transcriptional activity of ERα and the effect of furanodienone on the luciferase activity was compared to that of E2 control [Lee et al., 2005; Balan et al., 2006].

TRANSIENT TRANSFECTION OF siRNA

Control siRNA and ERα siRNA were purchased from Santa Cruz Biotechnology, Inc. and RNA interference assay was done according to the manufacturer's protocol. Briefly, cells were seeded in six-well culture plates in DMEM medium supplemented with 10% fetal bovine serum and cells were transfected with siRNA using Lipofectamine 2000 (Invitrogen Corporation).

STATISTICAL ANALYSIS

All values are expressed as means ± SD. Student's *t*-test was carried out using Microsoft Excel software. Results were considered statistically significant at $P < 0.05$.

RESULTS

FURANODIENONE INHIBITED MCF-7, T47D, AND MDA-MB-231 CELLS PROLIFERATION

We first examined the effect of furanodienone on the growth of human breast cancer cells. After exposure to various concentrations of furanodienone for indicated durations, the relative protein mass of cells was determined using the SRB assay. As shown in Figure 1B–D, furanodienone could inhibit MCF-7, MDA-MB-231, and T47D cells proliferation in a dose (10–160 μM) dependent manner. MDA-

MB-231 cells were less sensitive to furanodienone than MCF-7 and T47D cells.

FURANODIENONE SELECTIVELY DOWN REGULATED THE EXPRESSION LEVEL OF THE ERα RECEPTOR SUBTYPE IN MCF-7 CELLS

MCF-7 is an estrogen-responsive breast cancer cell line which expresses ERα as well as ERβ. ERα responsiveness is generally associated with proliferation whereas dominance of ERβ responsiveness has been linked to anti-proliferation processes. We examined the expression levels of ERα and ERβ in furanodienone-treated cells. Results showed that after 24 h of treatment furanodienone down-regulated ERα mRNA (Fig. 2A) and protein (Fig. 2B) expression levels. At 40 μM furanodienone time-dependently decreased the protein level of ERα (Fig. 2C). On the other hand, ERβ protein level was not affected by furanodienone within the concentration range of 20–80 μM after 24 h of treatment (Fig. 2D).

FURANODIENONE TREATMENT INHIBITED E2-INDUCED PROLIFERATION OF MCF-7 CELLS

The growth of estrogen-responsive human breast cancer cell lines, such as MCF-7, is strongly stimulated by estrogens if cells are pre-conditioned in a steroid-deficient medium [Sundar et al., 2006]. MCF-7 cells were first grown in phenol red-free medium containing 5% charcoal dextran-stripped fetal bovine serum. Our results showed that furanodienone at 20–80 μM could dose-dependently inhibit 10 nM E2-stimulated cell proliferation in pre-conditioned MCF-7 cells (Fig. 3A).

FURANODIENONE TREATMENT DISRUPTED E2-INDUCED CELL CYCLE PROGRESSION

Flow cytometry was used to examine cell cycle progression of PI-stained MCF-7 cells. Cells were cultured for 48 h under the indicated conditions. As shown in Figure 3B,C, 10 nM E2 treatment reduced the number of cells in the G1 phase and caused a significant increase of S phase cells. Treatment with 20 μM furanodienone

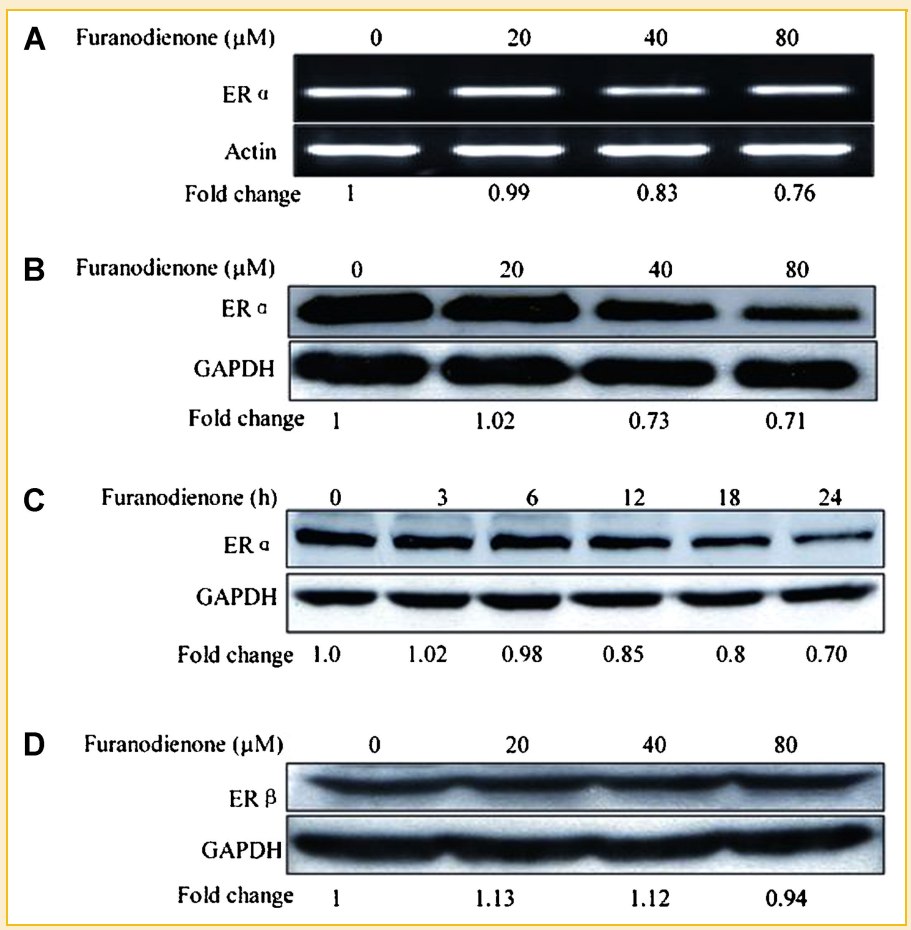

Fig. 2. Effects of furanodienone on the expression of ERα and ERβ in MCF-7 cells. A: Cells were treated with various concentrations of furanodienone for 24 h. Total RNA was isolated and mRNA level of ERα was measured by RT-PCR. Actin levels served as a loading control. B: Cells were treated with different concentrations of furanodienone for 24 h. Cell lysate was prepared and the protein level of ERα was determined by Western blotting. C: Cells were treated with 40 μM furanodienone for indicated durations and ERα protein level was examined by immunoblotting. D: Cells were treated with various concentrations of furanodienone for 24 h and ERβ protein was detected by Western blotting. Each experiment was repeated three times. The density of each band was quantified using the ImageJ 1.41o (NIH) and normalized to actin or GAPDH. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

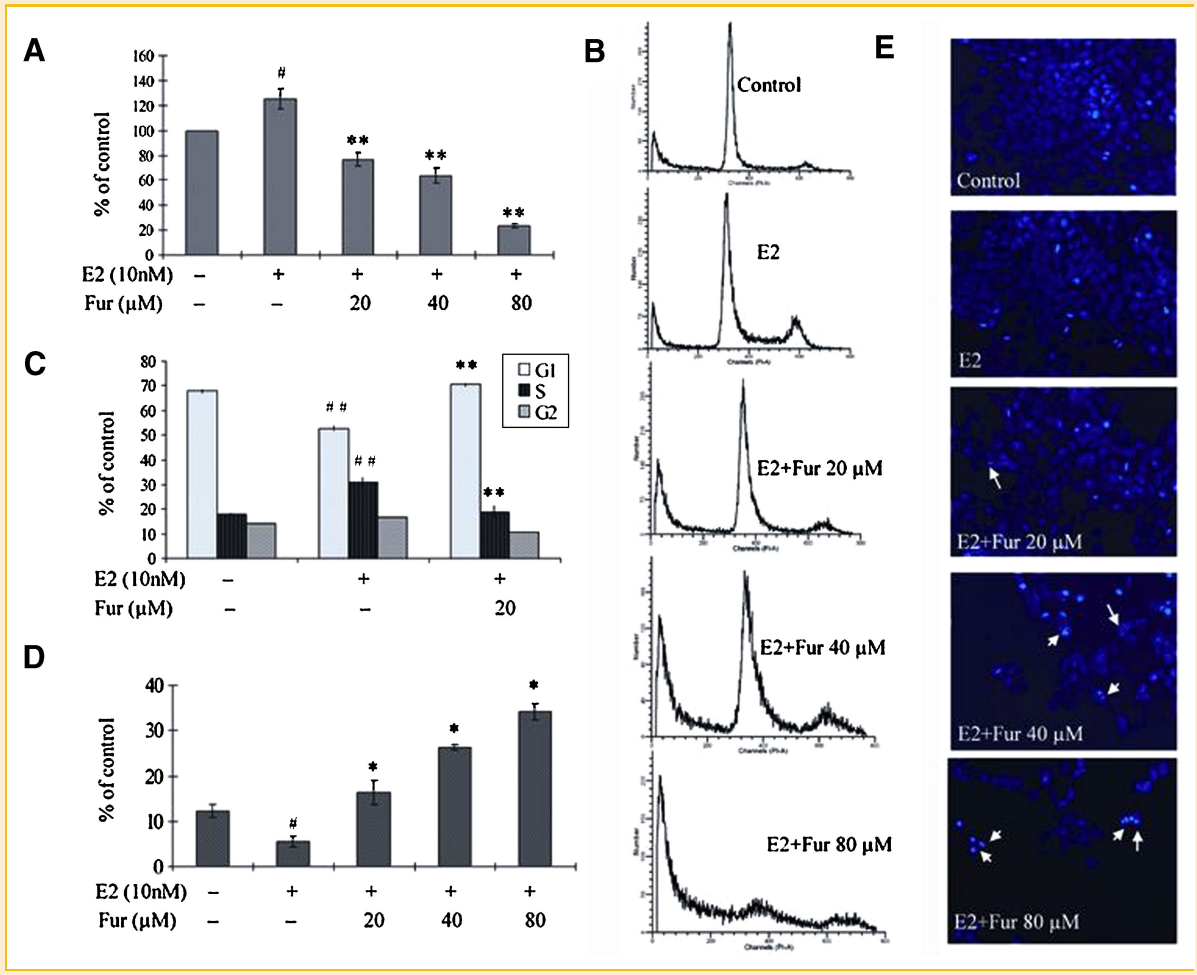

Fig. 3. Furanodienone inhibited E2-stimulated cell proliferation and cell cycle progression, and induced apoptosis in MCF-7 cells. A: Cells were pre-conditioned for 48 h in phenol red-free medium containing 5% charcoal dextran-stripped fetal bovine serum and were treated for another 48 h with various concentrations of furanodienone. Cell growth was assessed by SRB assay. $^{\#}P < 0.05$ versus the control; $^{**}P < 0.01$ versus E2 treatment. B: Representative flow cytometric results of three independent experiments. Cells were pre-conditioned for 48 h in phenol red-free medium supplemented with 5% dextran charcoal-stripped fetal bovine serum and treated as indicated. C: Quantification of cell cycle distribution from three independent experiments. $^{\#\#}P < 0.01$ versus the control; $^{**}P < 0.01$ versus E2 treatment. D: Sub-G1 phase cell populations from three independent experiments were quantified. $^{\#}P < 0.05$ versus the control; $^{*}P < 0.05$ versus E2 treatment. E: Representative morphological changes of three independent experiments. Cells were pre-conditioned for 48 h in phenol red-free medium supplemented with 5% dextran charcoal-stripped fetal bovine serum and treated with 10 nM E2 in the presence or absence of 20–80 μM furanodienone for 48 h. Arrows indicated apoptotic bodies after DAPI staining. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

blocked the E2-induced increase of S phase cells and the decrease of G1 phase cells. Furanodienone treatment also increased sub-G1 phase cell populations in a dose (20–80 μM) dependent manner in the presence of E2 (Fig. 3D).

APOPTOTIC MORPHOLOGICAL FEATURES WERE OBSERVED IN FURANODIENONE TREATED MCF-7 CELLS

To further investigate effects of furanodienone on the induction of apoptosis, DAPI staining was performed to observe morphological features. After treatment as indicated before, apoptotic bodies were observed in MCF-7 cells under a fluorescence microscope (Fig. 3E).

FURANODIENONE TREATMENT DISRUPTED E2-STIMULATED ESTROGEN RESPONSE ELEMENT PROMOTER ACTIVITY

To examine whether furanodienone can alter ERα signaling, MCF-7 cells were transfected with the ERE-Luc reporter gene and incubated with E2 in the absence or presence of furanodienone. Our results demonstrated that furanodienone at 20–80 μM could dose-dependently inhibit E2-induced estrogen responsive element (ERE)-driven reporter plasmid activity (Fig. 4).

FURANODIENONE SUPPRESSED ERα-TARGETED GENE EXPRESSION IN MCF-7 CELLS

Since furanodienone inhibited E2-mediated stimulation of ERE-Luc reporter, we further monitored the expression of three estrogen-targeted genes, namely c-Myc, cyclin D1, and Bcl-2, in MCF-7 cells. Total RNA and protein were extracted from E2-stimulated cells and RT-PCR and Western blot analyses were conducted. As expected, E2 induced c-Myc, cyclin D1, and Bcl-2 mRNA and protein expressions. The induction of the three proteins was inhibited by 24 h treatments of furanodienone (Fig. 5A,B).

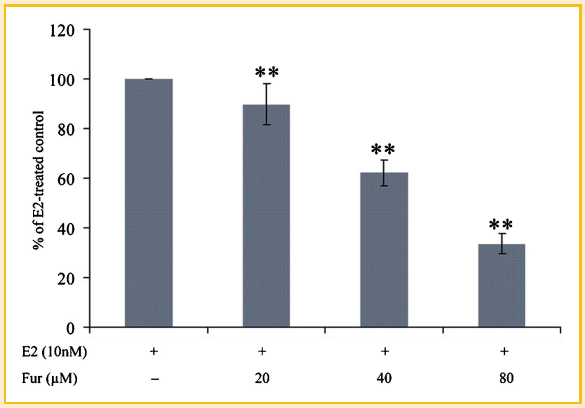

Fig. 4. Furanodienone blocked E2-stimulated estrogen responsive element promoter activity in MCF-7 cells. Four hours after transfection, cells were treated with 10 nM E2 and furanodienone (20–80 μM). Luciferase activity was monitored and normalized to β-galactosidase activity. The relative luciferase activity was averaged from three independent experiments each with triplicate wells. $^{**}P < 0.01$ versus E2 treated control.

ANTI-PROLIFERATION EFFECT OF FURANODIENONE ON MCF-7 CELLS WAS DEPENDENT ON ERα EXPRESSION

To examine whether ERα plays a role in the anti-proliferation activity of furanodienone, we studied the effect of furanodienone in cells of which ERα expression was down regulated by transient transfection of ERα-specific siRNA. Forty-eight hours after siRNA transfection ERα knockdown was verified with Western blot analysis (Fig. 6A). ERα-knockdown cells were treated for another 48 h with 20 or 40 μM furanodienone. Our results showed that growth of ERα-siRNA transfected and verified ERα-knockdown cells were more resistant to furanodienone inhibition (Fig. 6B). These results show that the anti-proliferation effect of furanodienone is dependent, at least in part, on ERα expression.

DISCUSSION

Breast cancer is the most common cancer and the second leading cause of women death worldwide [Jemal et al., 2008]. The female hormone estrogen and ERα play an important role in the etiology and progression of breast cancer. Estrogen induces cell proliferation and suppresses apoptosis through ERα-mediated genomic and non-genomic effects [Bjornstrom and Sjoberg, 2005; Blander, 2006]. About 70% breast cancers are ERα positive and depend on estrogens for growth [Platet et al., 2004]. Therefore, blocking estrogen-mediated actions has been one of the top priorities when considering treatment and prevention of ERα positive breast cancer. Currently, ERα transcription and signaling can be clinically blocked or reduced by drugs such as tamoxifen or fulvestrant, and estrogen levels can be reduced by aromatase inhibitors [Buzdar and Robertson, 2006]. However, not all ERα positive breast cancers respond to these drugs and it is of great value to develop new drugs targeting ERα signaling for the treatment of ERα positive breast cancer.

In this study, we observed that furanodienone significantly inhibited cell proliferation in human breast cancer MCF-7, T47D, and MDA-MB-231. ERα-negative MDA-MB-231 cells were less sensitive to furanodienone than ERα-positive MCF-7 and T47D cells. ERα belongs to the nuclear receptor superfamily and is one of the ligand-dependent transcription factors. Binding of estrogen to ERα causes conformational changes of the receptors and formation of receptor homo-complex. Following dimerization, the activated complex binds to estrogen-responsive elements in the promoter regions of ERα-dependent genes, and stimulates transcription of a variety of E2-responsive genes [Klinge, 2001]. Many of these gene products directly promote breast cancer cell proliferation and survival. Examples are the cell cycle regulators c-Myc and cyclin D1, and the anti-apoptotic factor Bcl-2 [Prall et al., 1998; Fiskus et al., 2007; Musgrove et al., 2008]. Results from this study demonstrated that furanodienone blocked E2 stimulation of estrogen responsive

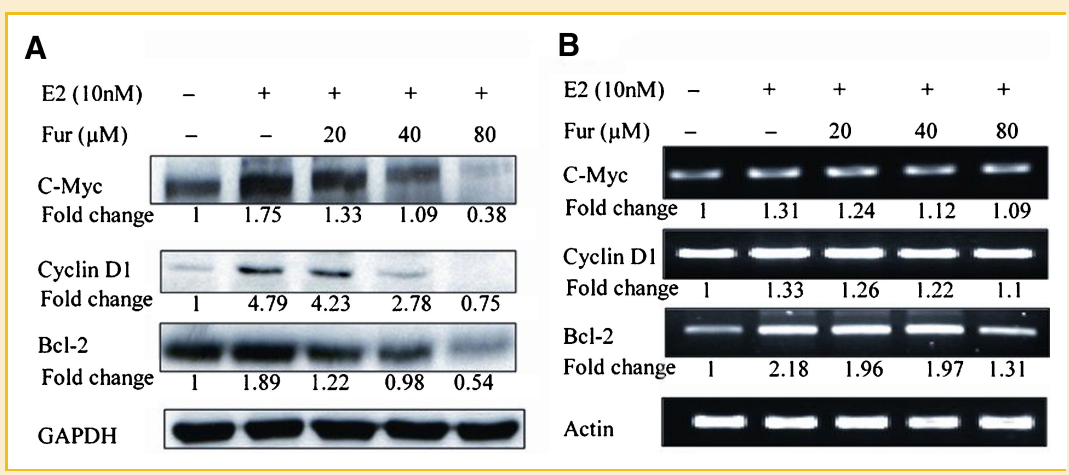

Fig. 5. Effect of furanodienone on the expression levels ERα-regulated genes in E2-stimulated MCF-7 cells. Cells were incubated with E2 and various concentrations of furanodienone for 24 h. A: Protein expression levels of cyclin D1, c-Myc, and Bcl-2 were detected by Western blotting. GAPDH was included as a loading control. B: The mRNA expression levels of cyclin D1, c-Myc, and Bcl-2 were detected by RT-PCR. Actin was included as a loading control. Each experiment was repeated three times. The density of each band was quantified using the ImageJ 1.41o (NIH) and normalized to actin or GAPDH.

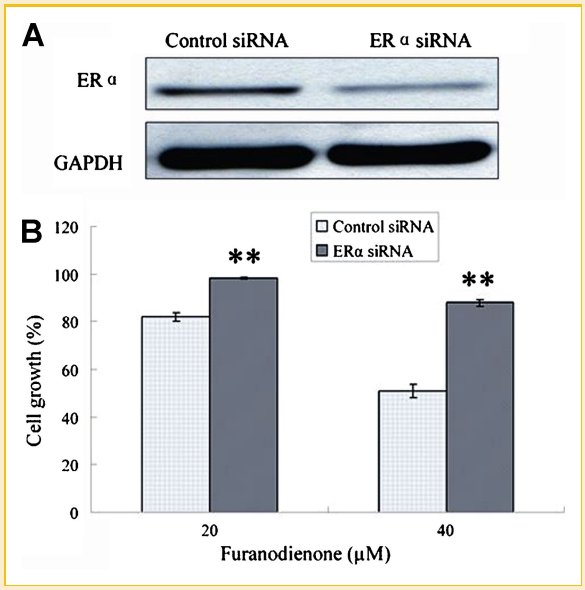

Fig. 6. ERα-knockdown in siRNA transfected MCF-7 cells modulated the growth inhibitory effect of furanodienone. A: Cells were treated with control siRNA or ERα siRNA for 96 h. Western blot analysis was performed to verify down-regulated expression level of ERα. GAPDH was used as a loading control. B: Effects of furanodienone on the growth of cells transfected with control or ERα siRNA. Forty-eight hours after siRNA transfection, cells were treated with furanodienone at 20–40 μM for another 48 h. The growth-inhibitory effect of furanodienone on MCF-7 cells was assessed by SRB assay. Growth of ERα siRNA-transfected cells was compared to that of control siRNA-transfected cells. Data shown are means ± SD from three independent determinations. $^{**}P < 0.01$.

element (ERE)-driven reporter plasmid activity and ablated the E2-induced expression of c-Myc, Bcl-2, and cyclin D1, which effectively blocked E2-stimulated cell proliferation and cell cycle progression, and induced apoptosis.

To further support our hypothesis, we studied furanodienone action in ERα-knockdown MCF-7 cells and found that the anti-proliferation activity of furanodienone was dependent on ERα expression level. This suggests that the anti-cancer effect of furanodienone was regulated, at least in part, by inhibiting ERα signaling. We have found that furanodienone at 40–160 μM induced cell death in ERα-negative MDA-MB-231 cells as evidenced by flow cytometry detected of sub-G1 cells (Supplementary figure). Further studies would be needed to elucidate whether furanodienone exerted its effects through other members of nuclear receptor supergene family.

In summary, furanodienone decreased ERα protein and transcription levels. In addition, it prevented E2-stimulated ERE-driven reporter plasmid activity and ablated the E2-induced expression of c-Myc, Bcl-2, and cyclin D1, which could result in inhibition of cell cycle progression and cell proliferation, and induction of apoptosis. Knockdown of ERα by ERα-specific siRNA decreased the anti-proliferation activity of furanodienone in MCF-7 cells. Our findings provide a rational and scientific basis for the use of furanodienone and related compounds for further development of novel agents useful in chemoprevention and the treatment of ERα positive breast cancer.

ACKNOWLEDGMENTS

We thank Dr. Anfernee Tse for the generous gift of ERE-Luc construct. This work was supported by a Faculty Research Grant of Hong Kong Baptist University (FRG2/08-09/102).

REFERENCES

Arpino G, Wiechmann L, Osborne CK, Schiff R. 2008. Crosstalk between the estrogen receptor and the HER tyrosine kinase receptor family: Molecular mechanism and clinical implications for endocrine therapy resistance. Endocr Rev 29:217–233.

Balan KV, Wang Y, Chen SW, Chen JC, Zheng LF, Yang L, Liu ZL, Pantazis P, Wyche JH, Han Z. 2006. Proteasome-independent down-regulation of estrogen receptor-alpha (ERalpha) in breast cancer cells treated with 4,4′-dihydroxy-*trans*-stilbene. Biochem Pharmacol 72:573–581.

Bjornstrom L, Sjoberg M. 2005. Mechanisms of estrogen receptor signaling: Convergence of genomic and nongenomic actions on target genes. Mol Endocrinol 19:833–842.

Blander CL. 2006. Estrogens and breast cancer. N Engl J Med 354:1647–1648; author reply 1647–1648.

Buzdar AU, Robertson JF. 2006. Fulvestrant: Pharmacologic profile versus existing endocrine agents for the treatment of breast cancer. Ann Pharmacother 40:1572–1583.

Ding J, Feng Y, Wang HY. 2007. From cell signaling to cancer therapy. Acta Pharmacol Sin 28:1494–1498.

Fiskus W, Ren Y, Mohapatra A, Bali P, Mandawat A, Rao R, Herger B, Yang Y, Atadja P, Wu J, Bhalla K. 2007. Hydroxamic acid analogue histone deacetylase inhibitors attenuate estrogen receptor-alpha levels and transcriptional activity: A result of hyperacetylation and inhibition of chaperone function of heat shock protein 90. Clin Cancer Res 13:4882–4890.

Houghton P, Fang R, Techatanawat I, Steventon G, Hylands PJ, Lee CC. 2007. The sulphorhodamine (SRB) assay and other approaches to testing plant extracts and derived compounds for activities related to reputed anticancer activity. Methods 42:377–387.

Jemal A, Siegel R, Ward E, Hao Y, Xu J, Murray T, Thun MJ. 2008. Cancer statistics, 2008. CA Cancer J Clin 58:71–96.

Jordan VC, Brodie AM. 2007. Development and evolution of therapies targeted to the estrogen receptor for the treatment and prevention of breast cancer. Steroids 72:7–25.

Klinge CM. 2001. Estrogen receptor interaction with estrogen response elements. Nucleic Acids Res 29:2905–2919.

Lee SO, Nadiminty N, Wu XX, Lou W, Dong Y, Ip C, Onate SA, Gao AC. 2005. Selenium disrupts estrogen signaling by altering estrogen receptor expression and ligand binding in human breast cancer cells. Cancer Res 65:3487–3492.

Li X, Wang G, Zhao J, Ding H, Cunningham C, Chen F, Flynn DC, Reed E, Li QQ. 2005. Antiproliferative effect of beta-elemene in chemoresistant ovarian carcinoma cells is mediated through arrest of the cell cycle at the G2-M phase. Cell Mol Life Sci 62:894–904.

Ma E, Wang X, Li Y, Sun X, Tai W, Li T, Guo T. 2008. Induction of apoptosis by furanodiene in HL60 leukemia cells through activation of TNFR1 signaling pathway. Cancer Lett 271:158–166.

Makabe H, Maru N, Kuwabara A, Kamo T, Hirota M. 2006. Anti-inflammatory sesquiterpenes from *Curcuma zedoaria*. Nat Prod Res 20:680–685.

Massarweh S, Schiff R. 2006. Resistance to endocrine therapy in breast cancer: Exploiting estrogen receptor/growth factor signaling crosstalk. Endocr Relat Cancer 13(Suppl 1): S15–S24.

Morandi P. 2005. Advances in the treatment of breast cancer. Womens Health (Lond Engl) 1:359–363.

Musgrove EA, Sergio CM, Anderson LR, Inman CK, McNeil CM, Alles MC, Gardiner-Garden M, Ormandy CJ, Butt AJ, Sutherland RL. 2008. Identification of downstream targets of estrogen and c-myc in breast cancer cells. Adv Exp Med Biol 617:445–451.

Platet N, Cathiard AM, Gleizes M, Garcia M. 2004. Estrogens and their receptors in breast cancer progression: A dual role in cancer proliferation and invasion. Crit Rev Oncol Hematol 51:55–67.

Prall OW, Rogan EM, Musgrove EA, Watts CK, Sutherland RL. 1998. c-Myc or cyclin D1 mimics estrogen effects on cyclin E-Cdk2 activation and cell cycle reentry. Mol Cell Biol 18:4499–4508.

Ricote M, Garcia-Tunon I, Fraile B, Fernandez C, Aller P, Paniagua R, Royuela M. 2006. P38 MAPK protects against TNF-alpha-provoked apoptosis in LNCaP prostatic cancer cells. Apoptosis 11:1969–1975.

Srinivasan S, Koduru S, Kumar R, Venguswamy G, Kyprianou N, Damodaran C. 2009. Diosgenin targets Akt-mediated prosurvival signaling in human breast cancer cells. Int J Cancer 125:961–967.

Sundar SN, Kerekatte V, Equinozio CN, Doan VB, Bjeldanes LF, Firestone GL. 2006. Indole-3-carbinol selectively uncouples expression and activity of estrogen receptor subtypes in human breast cancer cells. Mol Endocrinol 20:3070–3082.

Tanaka K, Kuba Y, Ina A, Watanabe H, Komatsu K. 2008. Prediction of cyclooxygenase inhibitory activity of Curcuma rhizome from chromatograms by multivariate analysis. Chem Pharm Bull (Tokyo) 56:936–940.

Tse AK, Wan CK, Shen XL, Zhu GY, Cheung HY, Yang M, Fong WF. 2007a. 1,25-Dihydroxyvitamin D3 induces biphasic NF-kappaB responses during HL-60 leukemia cells differentiation through protein induction and PI3K/Akt-dependent phosphorylation/degradation of IkappaB. Exp Cell Res 313:1722–1734.

Tse AK, Wan CK, Zhu GY, Shen XL, Cheung HY, Yang M, Fong WF. 2007b. Magnolol suppresses NF-kappaB activation and NF-kappaB regulated gene expression through inhibition of IkappaB kinase activation. Mol Immunol 44:2647–2658.

Wang G, Li X, Huang F, Zhao J, Ding H, Cunningham C, Coad JE, Flynn DC, Reed E, Li QQ. 2005. Antitumor effect of beta-elemene in non-small-cell lung cancer cells is mediated via induction of cell cycle arrest and apoptotic cell death. Cell Mol Life Sci 62:881–893.

Wang W, Yang S, Su Y, Xiao Z, Wang C, Li X, Lin L, Fenton BM, Paoni SF, Ding I, Keng P, Okunieff P, Zhang L. 2007. Enhanced antitumor effect of combined triptolide and ionizing radiation. Clin Cancer Res 13:4891–4899.

Xiao Y, Yang FQ, Li SP, Gao JL, Hu G, Lao SC, Conceicao EL, Fung KP, Wangl YT, Lee SM. 2007. Furanodiene induces G2/M cell cycle arrest and apoptosis through MAPK signaling and mitochondria-caspase pathway in human hepatocellular carcinoma cells. Cancer Biol Ther 6:1044–1050.