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Tryptanthrin inhibits *MDR1* and reverses doxorubicin resistance in breast cancer cells

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Abstract

Development of agents to overcome multidrug resistance (MDR) is important in cancer chemotherapy. Up to date, few chemicals have been reported to down-regulate *MDR1* gene expression. We evaluated the effect of tryptanthrin on P-glycoprotein (P-gp)-mediated MDR in a breast cancer cell line MCF-7. Tryptanthrin could depress overexpression of *MDR1* gene. We observed reduction of P-gp protein in parallel with decreases in mRNA in MCF-7/adr cells treated with tryptanthrin. Tryptanthrin suppressed the activity of *MDR1* gene promoter. Tryptanthrin also enhanced interaction of the nuclear proteins with the negatively regulatory CAAT region of *MDR1* gene promoter in MCF-7/adr. It might result in suppression of *MDR1* gene. In addition, tryptanthrin decreased the amount of mutant p53 protein with decreasing mutant p53 protein stability. It might contribute to negative regulation of *MDR1* gene. In conclusion, tryptanthrin exhibited MDR reversing effect by down-regulation of *MDR1* gene and might be a new adjuvant agent for chemotherapy.

Keywords: Breast cancer; Doxorubicin; MCF-7; *MDR1*; MDR reversal; Multidrug resistance; p53; Tryptanthrin

Multidrug resistance (MDR) is a major cause of failure of cancer chemotherapy. MDR can be caused by the transport proteins of ATP-binding cassette (ABC) family [1,2]. These membrane proteins actively transport drugs out of cells, resulting in a decrease of intracellular drug concentration. In humans, the first identified ABC transporter is P-glycoprotein (P-gp, also named as MDR1) [3]. In tumor cell lines, transfection of the *MDR1* gene confers resistance and expression of P-gp correlates with the degree of resistance [4].

Although P-gp is expressed normally in many different tissues, the physiologic function of the protein remains under active investigation [5]. Many studies have provided evidence implicating complex mechanisms for transcriptional regulation of the *MDR1* gene in human cancer cells [6]. The human *MDR1* gene promoter contains a number of recognition sites for SP1, NF-Y, and YB-1 transcription factors which can up-regulate *MDR1* promoter activity [7,8]. It was reported that a protein complex was involved in the negative regulation of *MDR1* promoter by interacting with the CAAT region of this promoter in MCF-7/wt, but not in MCF-7/adr cells [9]. In addition, inactivation of p53 by mutations can cause resistance to doxorubicin *in vivo* and the mutational status of p53 might be associated with drug resistance in human tumors [10,11].

One of the effective ways to overcome P-gp-mediated drug resistance is either to block its drug pump function or to inhibit its expression. Calcium channel blockers such as verapamil have been attempted to reverse P-gp-mediated MDR by blocking drug-pumping function of P-gp. However, these agents exhibit dose-limiting side effects that severely restrict their clinical utility [12]. To date, an effective and safe P-gp inhibitor has not been described.

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Tryptanthrin, a bioactive ingredient of *Polygonum tinctoriu* as a member of the Indigo plant family, is a quinazoline derivative and has been reported to have various biological activities, such as anti-microbial [13], anti-tumor [14] and anti-inflammatory activities [15]. In this report, we found tryptanthrin exhibited MDR-reversing activity and demonstrated the molecular mechanism of tryptanthrin.

Methods

Chemicals, cell lines and cell culture. Synthesis of tryptanthrin (6,12-dihydro-6,12-dioxoindolo-[2,1-b]qinazoline) (Fig. 1A) and its derivatives was as described [16]. MCF-7 human breast cancer cell line and its MDR variant MCF-7/adr were provided by Dr. Chih-Hsin Yang (National Taiwan University Hospital, Taiwan). Cells were maintained in DMEM with 10% fetal calf serum and 100 ng/ml of penicillin and streptomycin (Invitrogen, Carlsbad, CA) at 37 °C in 5% CO_2. MCF-7/adr cells were grown in the medium containing 6–8 μg/ml of doxorubicin, deprived 1 week before assays.

MTT assay. Cells were seeded into 96-well plates at a density of 5×10^3/well. Cytotoxicity of doxorubicin, tryptanthrin, verapamil and their combinations in MCF-7/wt and MCF-7/adr cells was analyzed by MTT assay after incubation of cells with these compounds for 5 days.

Determination of MDR1 amplification state. Genomic DNA was isolated from MCF-7/wt and MCF-7/adr cells treated with or without tryptanthrin. Two primers were designed to generate a DNA fragment (NT 079595.2: 12412842–12412662, 181 bp) between exon 14 and intron 14 of *MDR1* gene. Forward primer 5′-TGGGGCTTTTAGTGTTGGAC-3′ Backward primer 5′-TGTGGAGAGCTGGATAAAGTGA-3′.

Real-time quantitative PCRs were performed on the LightCycler™ system (Roche Diagnostics) using SYBER green I kits as recommended by the manufacturer.

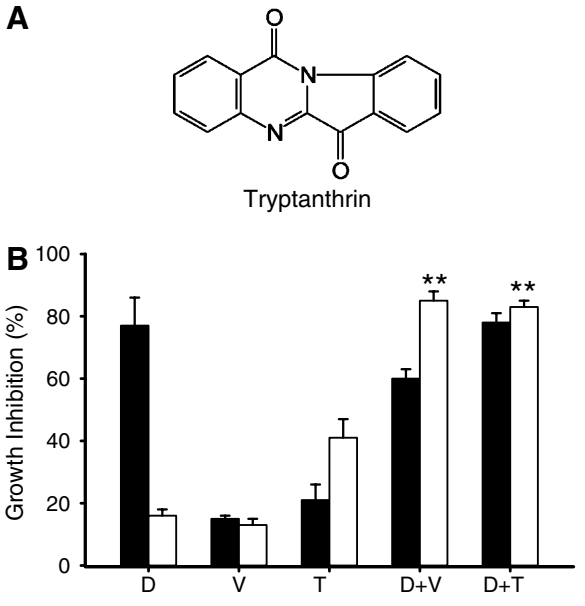

Fig. 1. (A) Structure of tryptanthrin. (B) Effect of tryptanthrin (T) or verapamil (V) combined with doxorubicin (D) on the growth of MCF-7/wt (■) or MCF-7/adr (□). The percentage of growth inhibition was determined by a MTT assay. Data were presented as means ± SD from three independent experiments. **Significantly different from D group ($p < 0.01$).

Determination of gene expression by RT-PCR and real-time PCR. MDR1 expression was assayed as described [17]. Total RNA was isolated from MCF-7/wt or MCF-7/adr cells and subjected to RT-PCR and real-time PCR. The primers used for PCR were shown below: (1) MDR1 primers sense 5′-CCCATCATTGCAATAGCAGG-3′ antisense 5′-GTTCAAACTTCTGCTCCTGA-3′. (2) GAPDH primers sense 5′-AGT CAACGGATTTGGTCGTA-3′ antisense 5′-GGAACATGTAAACCA TGTAG-3′.

The PCR products were electrophoresized and quantified by ImgaeMaster® VDS (GE Healthcare, Little Chalfont, Buckinghamshire, UK).

Real-time PCRs were performed on the LightCycler™ system (Roche Diagnostics, Basel, Switzerland) using FastStart DNA Master SYBER green I kits. Samples were amplified with a precycling hold at 95 °C for 10 min, followed by 50 cycles of denaturation at 95 °C for 10 s, 58 °C–63 °C annealing for 5–10 s and extension at 72 °C for 14 s. Primers used for real-time PCR were the same as those for RT-PCR. GAPDH was used as an internal control.

Additional three primers were used for real-time PCR to determine the expression of wild-type and mutant p53 in MCF-7/wt or MCF-7/adr cells. Primers 1 and 2 were used for amplification of the wild-type p53 gene (103–367, 265 bp). Primers 1 and 3 were used for the mutant p53 gene (103–406 with a 21 bp deletion, 283 bp). Primer 1: sense 5′-TTGC CGTCCCAAGCAATGGAT-3′ primer 2: antisense 5′-AGTCACAG ACTTGGCTGTCCCAGA-3′ primer 3: p53 (21 deleted) antisense 5′-TG GCAAAACATCGTGCAAGTC-3′.

Determination of P-gp. MCF-7/adr cells were seeded into six-well plates at a density of 2×10^5/well in the presence of tryptanthrin at 10^{-6} M for 5 days. Total cellular proteins (80 μg/lane) were electrophoresized on 10% SDS–polyacrylamide gels. After electrophoresis, the proteins were transferred to a PVDF membrane (GE Healthcare, Little Chalfont, Buckinghamshire, UK). Cells were labeled with anti-MDR1 CD243 (monoclonal mouse IgG, Chemicon, Temecula, CA) and anti-β-actin AC15 (mouse monoclonal IgG_1, Sigma, St. Louis, MO). Immunoreactive bands were detected by anti-mouse HRP or anti-rabbit peroxidase-conjugated secondary antibody (Chemicon, Temecula, CA). Proteins were detected by ECL kit and measured by Molecular Dynamics (GE Healthcare, Buckinghamshire, UK).

Electrophoretic mobility shift assay (EMSA). EMSA was performed as described [9]. Briefly, MCF-7/wt and MCF-7/adr nuclear extracts were preincubated in poly(dI · dC)-containing binding buffer and then added with 5′-end [γ-^{32}P]ATP-labeled *MDR1* promoter fragments. Double-stranded (ds) oligonucleotides at 200-fold molar excess were used as cold competitors. The 5′-end (forward) DNA sequence of ds oligomers used was a CAAT-like motif: ATCAGCATTCAGTCAATCCGGGCC. The reaction mixtures were separated on 5% native polyacrylamide gels and subjected to autoradiography.

Construction of plasmids containing wild type MDR1 promoter. The sequences of the primers for plasmid construction are as follows [9]: primer 1: 5′-GCGCTAGCCTAGAGAGGTGCAACG-3′ (−198 to −182) primer 2: 5′-GCAGATCTGCGGCCTCTGCTTCTT-3′ (+28 to +43). The 241 bp *MDR1* promoter fragment (residues −198 to +43) was amplified by PCR using primers 1 and 2. The pGL3-Basic vector (Promega, Madison, WI) was digested with nheI and BglII (Promega, Madison, WI). The gel-purified PCR product was digested with NheI and BglII and cloned into the vector. The accuracy of pGL3-promoter vector was confirmed by direct sequencing (Misson Biotech, Taipei, Taiwan).

Transient transfection and luciferase assay. Cells (3×10^5 cells/well) were seeded into six-well plates and grown in 5 ml of DMEM with 10% FCS for 24 h before transfection. Using TransFast™ Transfection Reagent (Promega, Madison, WI), cells were transfected with 2.5–3 μg/well plasmid. Luciferase activities were measured using Bright-Glo™ Luciferase Assay System (Promega, Madison, WI). Luminescence was measured by using Berthold Microplate Luminometer (Berthold Technologies, Bad Wildbad, Germany).

Fluorescence melting curve analysis. DNA melting curves were obtained on the LightCycler (Roche Molecular Biochemicals) by measuring the fluorescence of SYBR Green I during a linear temperature

transition from 65 to 95 °C at 0.1 °C/s. A CAAT-like motif (−198 to −153) of *MDR1* promoter was used as a template. Fluorescence data were converted into melting peaks by the LightCycler software (Ver. 4.05) to determine DNA binding efficiency of tryptanthrin.

Sequencing of full-length p53 cDNA. Sequencing of full-length p53 cDNA was as described [18]. The primers used for amplifying p53 cDNA fragments, overlapping the full-length p53 coding sequence, were as follows: codons 1–148: 5′-ATGGAGGAGCCGCAGTCA-3′ and 5′-ATC AACCCACAGCTGCACAGGG-3′, codons 118–353: 5′GGGACAGCC AAGTCTGTGACT-3′ and 5′-CCTGGGCATCCTTGAGTT-3′, codons 253-393: 5′-ACCATCATCACACTGGAAGACTCC-3′ and 5′-ATGT CAGTCTGAGTCAGG-3′.

PCR products were sequenced by Misson Biotech (Taipei, Taiwan).

Pulse-chase labeling with [^{35}S]-methionine and immunoprecipitation. The p53 Ab3 mouse monoclonal IgG_1 antibody (Calbiochem, San Diego, CA) reacts with only mutant p53 protein under non-denaturing conditions but reacts with both mutant and wild form of p53 under denaturing conditions. The half-life of the mutant p53 proteins in MCF-7/adr cells was determined by pulse-chase labeling with [^{35}S]-methionine followed by immunoprecipitation [18]. For pulse-chase labeling, MCF-7/adr cells of 70–80% confluence were incubated in 5 ml of the methionine-free DME medium containing 15 mCi/ml [^{35}S]-methionine for 2 h at 37 °C in 5% CO_2. The medium was removed and the cells were washed three times with DMEM medium containing L-methionine and 80 mg/ml cycloheximide (CHX, Sigma, St. Louis, MO), followed by incubation for 0–4 h. The labeled cells were washed, dissolved in 0.5 ml of cell lysis reagent (20 mM pH7.5 Tris–HCl, 125 mM NaCl, 1% Triton X-100, 1 mM $MgCl_2$, 1 mM PMSF, 25 mM α-glycerophosphate, 50 mM NaF, 100 μM $NaVO_3$, 10 μg/ml leupeptin and 10 μg/ml aprotinin) and then incubated with the p53 Ab3 (1 mg/ml) at 4 °C for 1 h. The immune complexes were removed by 250 μl Sepharose A at 48 °C for 1 h. They were dissolved in 100 μl of sample buffer and separated on a 10% SDS–polyacrylamide gel. After staining and destaining, the gels were dried and exposed to Kodak-XAR-5 film.

Statistical analysis. All results are presented as means ± SD. Comparisons between groups were analyzed via *t*-tests (two-sided). Probability values of $p < 0.05$ were considered statistically significant.

Results

MDR-reversing effect of tryptanthrin

Cells were incubated with 10^{-6} M doxorubicin and/or 10^{-6} M tryptanthrin for 5 days and subjected to MTT assays. Verapamil, an MDR-reversing agent, was used as a positive control for doxorubicin sensitization (Fig. 1B). Compared to MCF-7/wt cells, MCF-7/adr cell growth was only 20% inhibited upon 10^{-6} M doxorubicin treatment. Addition of 10^{-6} M tryptanthrin increased the cytotoxic effect of doxorubicin in MCF-7/adr cells, while 10^{-5} M, but not 10^{-6} M, verapamil reversed doxorubicin resistance in MCF-7/adr cells. It suggested that tryptanthrin is more potent than verapamil in terms of MDR-reversing effect.

Tryptanthrin does not alter amplification state of MDR1 gene

Inhibition of P-gp function may result from reduction of P-gp production, which may be attributed to changes in *MDR1* gene copies, mRNA production and protein amount. *MDR1* gene was amplified up to 100-folds in MCF-7/adr cells, compared to MCF-7/wt cells, using real-time PCR. However, treatment of tryptanthrin for 5 days did not alter amplification state of *MDR1* gene.

Tryptanthrin down-regulates expression of MDR mRNA and protein

Overexpression of *MDR1* gene and protein has been related to MDR phenotype [2]. Expression of *MDR1* mRNA was assessed by RT-PCR (Fig. 2A), and confirmed by real-time PCR (Fig. 2B). *MDR1* mRNA was expressed in MCF-7/adr cells, but not in MCF-7/wt cells. We examined if tryptanthrin could down-regulate expression of *MDR1* gene in MCF-7/adr cells. Results showed that 10^{-6} M tryptanthrin decreased *MDR1* gene expression in MCF-7/adr cells on the fifth day, in parallel with reduction of protein expression of *MDR1* (Fig. 2C).

Tryptanthrin down-regulates MDR1 expression by interfering binding of CAAT motif with nuclear transcription factors

Many studies have provided evidence implicating complex mechanisms for transcriptional regulation of the

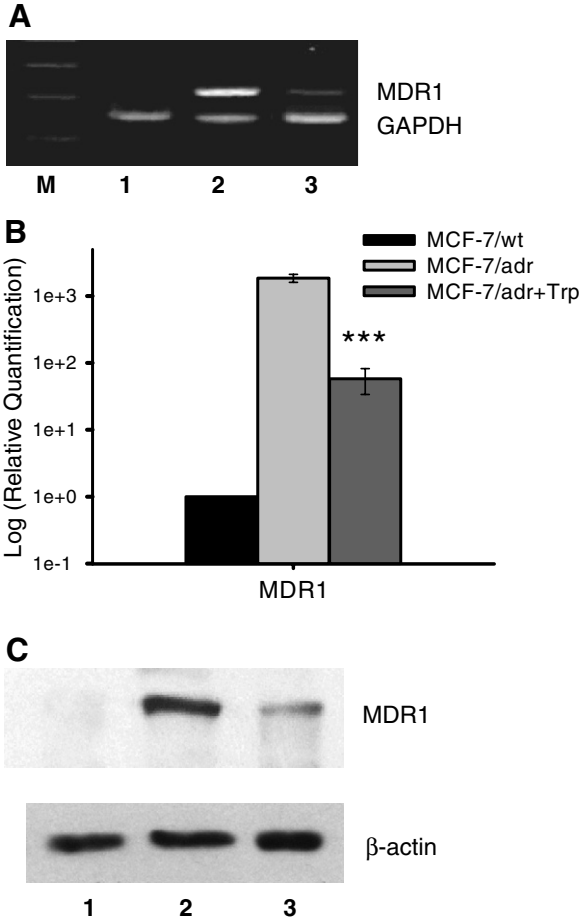

Fig. 2. Gene expression and protein synthesis of *MDR1* were suppressed in MCF-7/adr cells treated with 10^{-6} M tryptanthrin (Trp). (A) *MDR1* mRNA levels were detected in MCF-7/wt (lane 1) and MCF-7/adr (lane 2) cells and MCF-7/adr cells with Trp (lane 3). (B) Real-time PCR was performed to confirm the results in (A). Data are represented as means ± SD ($n = 3$). $^{***}p < 0.001$ is compared with untreated MCF-7/adr cells. (C) Protein levels of MCF-7/wt (lane 1), MCF-7/adr (lane 2) and MCF-7/adr with tryptanthrin (lane 3) were detected using immunoblotting. β-Actin was used as the loading control.

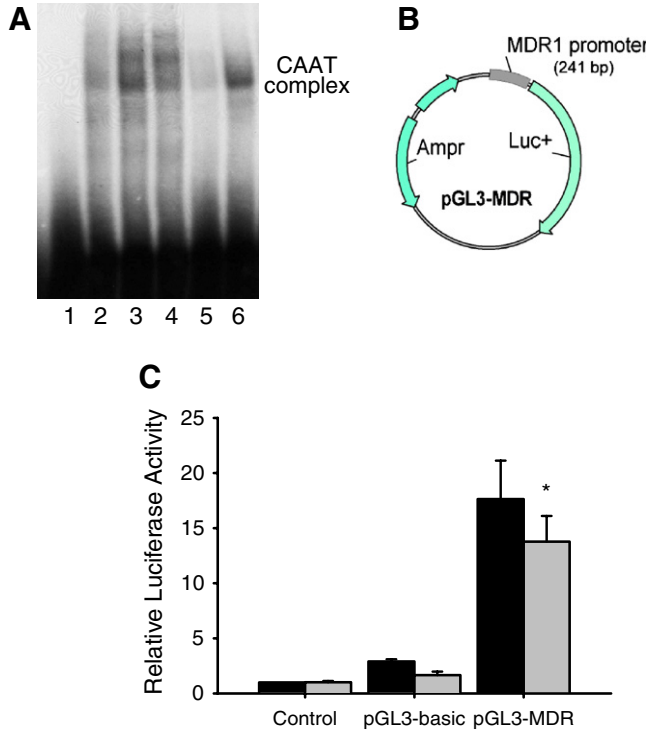

Fig. 3. Effect of tryptanthrin on *MDR1* gene promoter. (A) Analysis of the interaction between nuclear protein complex and CAAT region in *MDR*1 gene promoter by gel mobility shift. Lane 1, unbound probe; lane 2, 5′-end-labeled ds CAAT-like oligomers incubated with MCT-7/wt nuclear extracts in the presence of competitor cold probe; lane 3, MCF-7/wt alone; lane 4, MCF-7/wt with tryptanthrin; lane 5, MCF-7/adr alone; lane 6, MCF-7/adr with tryptanthrin. (B) Construction of pGL3-MDR. The 241 bp *MDR*1 promoter DNA fragment was cloned into the pGL3-basic vector at the upstream region of the luciferase gene. (C) Analysis of *MDR1* promoter activity by luciferase assay in MCF-7/adr cells treated with (□) or without (■) 10^{-6} M tryptanthrin. Data are represented as means ± SD ($n = 4$). $^{*}p < 0.05$ represents significantly different from cells without tryptanthrin.

MDR1 gene in human cancer cells. Since we have demonstrated that tryptanthrin could inhibit expression of *MDR1* mRNA, it is interesting to examine whether tryptanthrin has any effect on *MDR1* promoter. Electrophoretic mobility shift assay was performed using a 5′-end labeled ds CAAT-like oligomers as a probe with nuclear extracts from MCF-7/wt or MCF-7/adr. One specific protein complex interacting with the probe (Fig. 3A, lane 3) was detected in MCF-7/wt cells, while no protein/DNA complex was observed in MCF-7/adr cells (Fig. 3A, lane 5). Interestingly, the protein/DNA complex apparently reappeared in MCF-7/adr cells treated with 10^{-6}M tryptanthrin (Fig. 3A, lane 6). It suggested that tryptanthrin rescued the binding of nuclear proteins with CAAT region of the *MDR1* gene promoter in MCF-7/adr cells, which might contribute to the inhibition of *MDR1*expression.

Analysis of MDR1 promoter activity in MCF-7/adr cells

To determine whether the CAAT segment of the *MDR1* promoter was interfered with tryptanthrin, wild-type *MDR1* promoter (residues −198 to +43, 241 bp) DNA fragment was cloned into a luciferase-expressing pGL3-basic vector at the upstream region of the luciferase gene to construct pGL3-MDR (Fig. 3B). The activity of the *MDR1* promoter was measured as a function of luciferase activity. A 15-folds increase of luciferase activity was detected in MCF-7/adr cells transiently transfected with pGL3-MDR, compared to that in MCF-7/adr transiently transfected with pGL3-basic vector (Fig. 3C). After added with 10^{-6} M tryptanthrin for 5 days, luciferase activity moderately decreased (about 20%) in MCF-7/adr cells transfected with pGL3-MDR ($p < 0.05$). It seems that direct effect of tryptanthrin on CAAT segment of *MDR1* promoter may partly contribute to its MDR-reversing effect.

It is interesting to know if tryptanthrin binds to *MDR1* promoter. Real-time PCR was performed to determine the melting peak of a CAAT segment of *MDR1* promoter affected in the presence of typtanthrin. There was a 6.7 °C drop of melting point upon tryptanthrin treatment, indicating the binding of tryptanthrin to the CAAT motif.

The effect of tryptanthrin on the expression of p53 in MCF-7/wt and MCF-7/adr cells

Mutations in p53 gene have been reported to play an important role on development of resistance to DNA damaging agents in several human cancer cell lines. Therefore, we attempted to understand if the status of p53 in MCF-7/wt and MCF-7/adr cells was related to the effect of tryptanthrin in these cells. We first characterized the full-length sequence of the p53 genes in MCF-7/adr and MCF-7/wt cells. Results showed that MCF-7/wt cells contained wild-type p53 sequence, consistent to the NIH p53 sequence NM000546. In contrast, we detected a 21 bp deletion in one allele of the p53 gene at upstream end of exon 5, spanning codons 127–133, in MCF-7/adr cells.

The p53 Ab-3 mouse monoclonal antibody reacts with only mutant p53 protein under non-denaturing conditions but it reacts with both mutant and wild type p53 proteins under denaturing conditions. We examined the status of mutant p53 protein in MCF-7/adr cells treated with or without tryptanthrin. Results showed overexpression of mutant p53 protein in MCF-7/adr cells without tryptanthrin treatment (Fig. 4A, lane 2). With 10^{-6} M tryptanthrin, expression of mutant p53 protein in MCF-7/adr cells decreased (Fig. 4A, lane 3). To study if inhibition of p53 gene transcription resulted in decrease of mutant p53 protein in MCF-7/adr cells with tryptanthrin, p53 mRNA levels were measured by real-time PCR (Fig. 4B). Results showed that total p53 mRNA levels did not importantly change in MCF-7/adr cells before and after tryptanthrin treatment. Furthermore, expression of mutant p53 mRNA was not significantly different in MCF-7/adr cells treated with and without tryptanthrin. These results suggested that decrease of mutant p53 protein in MCF-7/adr cells with tryptanthrin was not controlled at transcriptional level,

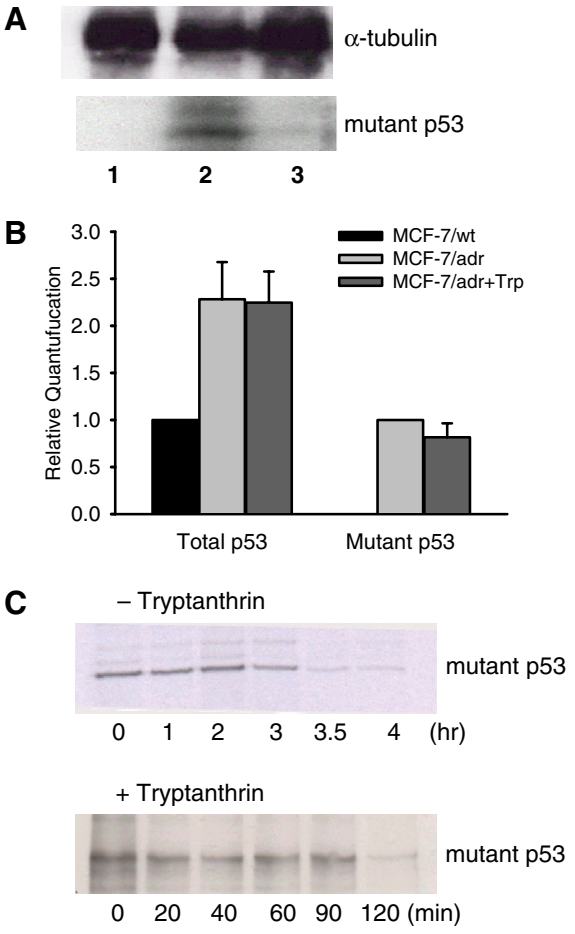

Fig. 4. Effect of tryptanthrin on p53 gene and protein expression in MCF-7/adr cells. (A) Levels of mutant p53 protein in MCF-7/wt (lane 1), MCF-7/adr alone (lane 2) and with 10^{-6} M tryptanthrin (lane 3) were detected by immunoprecipitation. (B) p53 gene expression in MCF-7/wt and MCF-7/adr cells was detected by real-time PCR. (C) Mutant p53 protein stability was examined by pulse-chase labeling with [^{35}S]-methionine.

but probably by post-transcriptional mechanisms such as decreased protein stability. To investigate the hypothesis, we examined stability of mutant p53 protein. Half-life of mutant p53 protein in MCF-7/adr before and after tryptanthrin treatment was measured by pulse-chase/immunoprecipitation (Fig. 4C). The half-life of mutant p53 protein in MCF-7/adr cells without tryptanthrin treatment was as long as 3 h and it was almost degraded after 4 h. In contrast, the half-life of mutant p53 protein in MCF-7/adr cells with tryptanthrin shortened to less than 90 min. It suggested that tryptanthrin altered mutant p53 protein stability and reduced its half-life.

Discussion

Resistance to cancer chemotherapy remains a major cause of treatment failure. Many attempts, such as inhibition of MDR-related genes, to overcome MDR have been proposed. Inhibition of *MDR1* gene function, either by blocking P-gp function or inhibiting *MDR1* gene expression has been one of the most extensive studies. Up to date, there has been few report concerning about a new class of chemicals which are able to down-regulate *MDR1* gene expression instead of just blocking P-gp transporter function.

Tryptanthrin and derivatives have been reported to exhibit anti-tumor effects. When we examined a series of quinazoline derivatives for their anti-tumor effects, we found out tryptanthrin displayed additive effect of cytotoxicity of doxorubicin in MCF-7/adr cells.

Our results showed the MDR reversing effect of tryptanthrin mostly resulted from inhibition of *MDR1* gene expression, and then inhibition of P-gp production. Since gene amplification has been frequently observed in cancers and *in vitro* drug-resistant models [19], it should be examined for the possibility of changes in DNA levels upon tryptanthrin treatment. Using real-time PCR to assess genomic DNA, we found that tryptanthrin did not modulate the copy number of *MDR1* gene in MCF-7/adr cells. We further demonstrated that tryptanthrin inhibited *MDR1* gene expression partly through acting on *MDR1* promoter.

Gel mobility shift experiments demonstrated that the interaction of nuclear protein complex with CAAT oligomers was found in MCF-7/wt cells, but not in MCF-7/adr cells. It is assumed that nuclear protein complex may have inhibitory effect on *MDR1* promoter in MCF-7/wt cells. It has been reported that a protein complex consisting of NF-κB/p65 and c-Fos might be involved in the negative regulation of *MDR1* promoter in MCF-7/wt [9]. On the other hand, some specific factors might exist and prevent nuclear proteins from binding CAAT region in MCF-7/adr. The specific factor(s) may exhibit this function through either blocking formation of nuclear proteins or directly bind the CAAT region of *MDR1* promoter, so that the interaction of nuclear proteins with CAAT region becomes unavailable. In the presence of tryptanthrin, the interaction of nuclear proteins with CAAT region reappeared. We hypothesize that tryptanthrin may block function of the specific factor(s), subsequently regain the interaction of nuclear proteins with CAAT region and inhibit *MDR1* gene expression. The evidence of tryptanthrin to bind DNA may suggest that it acts as a putative transcription factor itself for negative regulation on CAAT promoter of *MDR1* gene or as a modulator to restore the interaction of nuclear proteins with CAAT region in MCF/adr cells.

Furthermore, p53 has been related to regulation of *MDR1* gene. Wild-type p53 can inhibit *MDR1* gene expression while mutant p53 enhances *MDR1* gene expression [10,11]. Interestingly, MCF-7/wt cells contained wild-type p53 while MCF-7/adr contained mutant p53 in this study. It is reasonably suspected that p53 may play some important role in regulation of *MDR1* expression in MCF-7/wt and MCF-7/adr cells. We demonstrated that tryptanthrin had insignificant effect on the expression of p53 mRNA, but did depress mutant p53 protein level in MCF-7/adr cells. We further showed that decrease of mutant p53

protein level resulted from shortening of the half-life of mutant p53 protein. The result may in part contribute to down-regulation of *MDR1* gene expression.

Other attempts such as small interfering RNAs [20] have been made to selectively inhibit *MDR1* gene expression. However, none has been applied for clinical use yet. With this report, we demonstrated tryptanthrin exhibited MDR reversing effect specifically through down-regulation of *MDR1* gene expression.

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