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行政院國家科學委員會補助專題研究計畫成果報告

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行政院國家科學委員會專題研究計畫成果報告
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一、中文摘要

摘要: Terbinafine (TB)是目前市售抗黴菌藥物，廣泛應用於皮膚表淺黴菌感染症狀。有關它的抗癌作用在本實驗中首度探討。本實驗利用人類血癌細胞(HL 60)作爲研究材料，發現TB (5-20 μM) 可以造成細胞週期停滯於G0/G1 phase，與細胞週期相關之調控蛋白 p21/Cip1明顯地被誘發，同時CDK2,與CDK4 kinase活性亦大幅被抑制。另外，在高劑量(40-60 μM)TB處理下，可誘發HL 60細胞凋亡。 可以明顯地觀察到細胞內DNA斷片(Ladder)與流式細胞儀分析之sub-G1族群大量增加。與細胞凋亡相關的蛋白分析則發現caspases 3, 8和9的表現有被活化。我們的研究證實Eco對人類血癌細胞(HL 60)有明顯地抗癌作用，這樣的作用或許可以應用於臨床上地抗癌治療目的。
關鍵詞: Terbinafine， 抗癌作用， G0/G1 細胞週期停滯，細胞凋亡作用。

Abstract
Terbinafine (TB), a potent broad-spectrum anti-fungal agent, has been used in the treatment of superficial mycosis. However, little is known about its potential anti-tumor effect. In this study, we demonstrated that lower doses TB (5-20 μM) arrested human HL 60 cancer cells at the G0/G1 phase of the cell cycle. The protein levels of p21/Cip1, was significantly elevated while CDK2 and CDK4 kinase activity were significantly suppressed by Eco treatment in HL 60 cells. At higher doses (40-60 μM), TB induced HL 60 cells apoptosis evidenced by ladder formation in DNA fragmentation assay and sub-G1 peak in flow cytometry analysis. Western blot analysis showed that caspases 3, 8 and 9 were activated by high dose (40-60 μM) Eco treatment to the HL 60 cell. Our findings provide the novel mechanisms of antitumor effects of TB and such results may have significant applications for cancer chemotherapy.

Key words: Terbinafine, Anti-cancer, G0/G1 cell cycle arrest, Apoptosis.

二、緣由與目的

The occurrence of cancers in Taiwan has been increased strikingly during the last decade. It is the leading cause of mortality in Taiwan and is one of the world's most common malignancies. The total number of cancer patient is greatly increased in the 2002 with a high mortality as 26.05%. Therefore, fighting against cancer is an important global issue. The antifungal agents have been shown to exert anti-tumor effect. For example, ketoconazole has been used in the treatment of hormone-dependent prostate cancer [1-3]. Ketoconazole inhibited hepatic metastasis from a human pancreatic adenocarcinoma [4] and reduce the incidence of pulmonary metastases in the nude mice melanoma model [5]. As in our previous studies, the oral antifungal agents griseofulvin and ketoconazole have been demonstrated to suppress tumor cell growth *in vitro* and *in vivo* [6-9]. Griseofulvin significantly potentiated the anti-cancer effect of nocodazole, leading to cessation of tumor growth. These results suggested that combined administration of antifungal agent and chemotherapeutic agents might provide a novel therapy for colorectal cancer [7]. These findings suggested that some antifungal agents might be a candidate compound for anticancer treatment.
Terbinafine (TB), an antifungal imidazole compound, has been used in the treatment of vulvovaginal candidiasis [10, 11] and superficial fungal infection [12, 13]. TB and

fluconazole are azole antifungal agents, each with a broad spectrum of activity against both superficial and systemic mycosis. Although KT is very effective for this indication, its use is associated with adverse reactions which can be sufficiently severe to require discontinuation of therapy. TB has been shown to inhibit lipopolysaccharide-inducible nitric oxide synthase (iNOS) activity in rat aortic rings and cultured J774 murine macrophage cells [14] suggesting it exerted a potential anti-inflammatory effect. Finally, TB at dose of 1 μg/ml was found to reduce cell numbers, viability and survival, and DNA synthesis in the NS1 myeloma line [15].

Over the past few years, there has been increasing rTBgnition of the important role of cell death in determining tissue cell number and how a lack of cell death under appropriate physiologic conditions can contribute to cellular transformation and malignant cell growth. Cell death permits the selective elimination of excess cells, such as in morphogenesis and the immune system, and also permits the maintenance of tissue homeostasis. The majority of these cell deaths share a common morphology, including a reduction in cell volume, blabbing of the plasma membrane, chromatin aggregation, and finally fragmentation of DNA. This morphologically distinct form of cell death is called apoptosis.

In this manuscript, we demonstrate that TB-induced apoptosis in different human cancer cells. However, the effects of the TB-induced HL 60 cells apoptosis were the most interesting issue. The aim of this study was to evaluate the molecular mechanisms of the apoptotic effects induced by TB so that further mechanistic investigations of cytotoxicity in human HL 60 cancer cells could be performed. The major nuclear membrane protein (Poly-[ADP ribose] polymerase, *PARP*) degradation induced by TB was detected by Western-blot analysis. The alterations of gene expression associated with the cell cycle (*p53*), signal transduction (ERK) and apoptosis (*Caspase-3, bax,* and *bcl-2*) in human HL 60 cancer cells treated with TB was also investigated in this study. This study provides further evidences that the antifungal agent TB might have potential application for cancer chemotherapy.

三、研究報告內容

MATERIAL AND METHODS

Chemicals

Terbinafine, Ketoconazole, fluconazole, amphotericin B, and griseofulvin were purchased from the Sigma Chemical Co. (St. Louis, MO). Flucytosine was purchased from the Merck Co. The protein assay kit was purchased from the Bio-Rad Co. (Bio-Rad Labs., Hercules, CA).

Cell lines and cell culture. The cell line CCD-922SK (CRL 1828; American Type Culture Collection) was derived from normal human fibroblasts [9]. The HL 60 cell line was derived from human myeloid leukemia cells (59170; American Type Culture Collection). The cells were grown in RPMI 1640 for HL 60 cells; Eagle's minimal essential medium, EMEM, for human fibroblasts, supplemented with 10% fetal calf serum (FCS), penicillin (100 U/ml), streptomycin (100 mg/ml), and 0.3 mg/ml glutamine in a humidified incubator (37 ^{O}C, 5% CO_2). TB (Sigma Chemical Co., St. Louis, MO) was added at the indicated doses in 0.05% dimethylsulfoxide (DMSO). For control specimens, the same volume of DMSO was added in a final concentration of 0.05% (v/v) without TB.

Determination of cell viability. HL 60 cells and fibroblasts were treated with TB (10-30 μM). Cell viability was determined at the indicated times based on the trypan blue exclusion method. The viability percentage was calculated based on the percentage of unstained cells as described previously [6].

Determination of cell growth curve. Human HL 60 (1 x 10^5), human fibroblasts (1 x 10^5) cells were plated in 35-mm petri dishes. The next day, the medium was changed and TB (5-60 μM) was added. Control cells were

treated with DMSO in a final concentration of 0.05% (v/v). The incubation medium was renewed every day during the experiment. At the end of incubation, cells were harvested for cell count with a hemocytometer.

Cell synchronization, drug treatment, and flow cytometry analysis. At 24 h after plating of cells, cells were washed three times with phosphate-buffered saline (PBS) and then incubated with medium containing 0.04% FCS for additional 24 h. Under such conditions, cells were arrested in G0/G1 as determined by flow cytometry analysis [16]. After serum starvation, the low-serum (0.04% FCS) medium was removed and the cells were then challenged by addition of medium containing 10% FCS. TB solutions were prepared in a final concentration of 0.05% (v/v) DMSO. The cell cycle stages in the TB and DMSO-treated groups were measured by flow cytometry analysis. Cells were harvested and stained with propidium iodide (50 μg/ml) (Sigma Chemical Co., St. Louis, MO), and DNA content was measured using a FACScan laser flow cytometer analysis system (Becton–Dickinson, San Jose, CA); and 15,000 events were analyzed for each sample.

Western analysis. Western blotting analysis was performed as described previously [7]. Briefly, cell lysates were prepared, electrotransferred, immunoblotted with antibodies, and then visualized by incubating with the colorigenic substrates (nitroblue tetrazolium, NBT and 5-bromo-4-chloro-3-indolyl phosphate, BCIP) (Sigma Chemical Co., St. Louis, MO). The expression of GAPDH was used as the control for equal protein loading.

Analysis of DNA fragmentation. Analysis of DNA fragmentation was performed as previously described [17]. Briefly, the TB and DMSO-treated cells were seeded on 100-mm dishes. The DNA was extracted twice with equal volumes of phenol and once with chloroform-isoamyl alcohol (24:1 v:v), then precipitated with 0.1 volume of sodium acetate, pH 4.8, and 2.5 volumes of ethanol at -20 ^{O}C overnight, and finally centrifuged at 13,000 X g for 1 h. Genomic DNA was quantitated, and equal amounts of DNA sample in each lane were electrophoresed in a 2 % agarose gel. The DNA was visualized by ethidium bromide staining.

Immunoprecipitation, CDK2, and CDK4 kinase activity assay. The p21/Cip1-associated CDK-2 kinase activity was determined as described previously [18]. Briefly, using 2 μg anti-CDK2 antibody and 20 μl protein A agarose beads, the protein complexes were precipitated from 200 μg of protein lysates per sample as described above. Beads were washed twice with lysis buffer and then once with kinase assay buffer (50 mM Tris–HCl, pH 7.4, 10 mM MgCl2, and 1 mM DTT). Phosphorylation of the histone H1 was measured by incubating the beads with 40 μl of "hot" kinase solution [0.25 μl (2.5 μg) of histone H1, 0.5 μl of [γ-^{32}P]ATP, 0.5 μl of 0.1 mM ATP, and 38.75 μl of kinase buffer] for 30 min at 37°C. The reaction was stopped by boiling the samples in SDS sample buffer for 5 min. The samples were analyzed using 12% SDS–PAGE, and the gel was dried and subjected to autoradiography. Similarly, the CDK4 kinase activity was determined as described by previous report [18] with some modifications. Briefly, TB-treated cells were lysed in Rb lysis buffer (50 mM Hepes–KOH, pH 7.5, containing 150 mM NaCl, 1 mM EDTA, 2.5 mM EGTA, 1 mM DTT, 0.1% Tween 20, 10% glycerol, 80 mM β-glycerophosphate, 1 mM sodium fluoride, 0.1 mM sodium orthovanadate, 1 mM phenylmethylsulfonyl fluoride, and 10 μg/ml leupetin and aprotinin) and immunoprecipitated with 2 μg anti-CDK4 antibody. The protein complexes in beads were washed twice with Rb lysis buffer and then once with Rb kinase assay buffer (50 mM Hepes–KOH, pH 7.5, containing 2.5 mM EGTA, 10 mM β-glycerophosphate, 1 mM sodium fluoride, 0.1 mM sodium

orthovanadate, 10 mM $MgCl_2$, and 1 mM DTT). Phosphorylation of Rb was measured by incubating the beads with 40 μl of hot Rb kinase solution [0.25 μl (2 μg) Rb–GST fusion protein, 0.5 μl of [γ-^{32}P]ATP, 0.5 μl of 0.1 mM ATP, and 38.75 μl of Rb kinase buffer] for 30 min at 37°C. The reaction was stopped by boiling the samples in SDS sample buffer for 5 min. The samples were analyzed using 12% SDS–PAGE, and the gel was dried and subjected to autoradiography.

RESULTS

Antifungal agents-Induced Apoptosis in Human HL 60 Cancer Cell Lines

The HL 60 cells treated with different antifungal agents (25 μM) exhibited morphological changes were accompanied by progressive internucleosomal degradation of DNA to yield a ladder of DNA fragments (Fig. 1). Our results confirmed the occurrence of apoptotic cell death after different antifungal agent exposure. As is shown in Fig.1, after exposure of HL 60 cells to TB (25 μM), both the attached and detached cells were harvested at 24 hr after drug treatment. In each case, nucleosomal DNA ladders typical of apoptosis were visible on agarose gel after staining with ethidium bromide (Fig.1). In such a condition, we found that TB was the more potentially effective in induction of the HL 60 cells apoptosis. Several additional human cancer cell lines were also examined for TB-induced apoptosis. However, DNA ladders were not clearly evident enough at 24 hr after all these human cancer cell lines were exposed to TB (25 μM) (data not shown). For these reasons, HL 60 cells were used as a cultured cell model for investigation of the molecular mechanisms of TB-induced apoptosis.

TB-Induced Cytotoxicity in HL 60 Cells

To investigate whether TB inhibited cancer cell growth, HL 60 and human normal fibroblast cells (CCD 922SK) were treated with different doses (1-30 μM) of TB for 1-5 days and the cell growth numbers were then determined (Fig. 2). TB at lower doses (5-10 μM) suppressed HL 60 cell proliferation while at higher dose (>20 μM) induced cell death (Fig. 2A). Interestingly, such effect was less profound on normal human fibroblasts treated with TB (Fig. 2B). We further demonstrated that the viability of HL 60 cells was significantly decreased when exposed to more than 10 μM TB for 24 hrs. In contrast, less significant influence of viability was observed in fibroblast treated with same dose of TB (data not shown). These results indicated that HL 60 cancer cells were more susceptible to TB treatment than in normal human fibroblasts.

TB Induced G0/G1 Cell Cycle Arrest and Apoptosis in HL 60 Cells

In order to determine the action of TB on a specific phase of cell cycle, the HL 60 cells were synchronized by switching them to media with 0.04% FCS for 24 hrs to render them quiescent. Cells were then returned to culture media containing 10% FCS with or without 25 μM TB (which started them all on a new cell cycle) and, at various times thereafter, they were harvested for flow cytometry analysis. Fig. 3A (right) showed that 25 μM TB induced a significant accumulation (>85%) of cells in the G0/G1 phase of the cell cycle compared to 0.05% DMSO control (left), suggesting that the growth inhibitory effect of TB was due to an arrest of DNA replication in the cell cycle. Figure 3B demonstrated the dose-dependent effect of TB on the cell cycle regulation. The results demonstrate that TB (5-20 μM) induced an accumulation (up to 85 %) of the HL 60 cells at the G0/G1 phase of the cell cycle. However, higher dose (40-60 μM) of TB induced HL 60 cell death as evidenced by sub-G1 peak in the flow cytometry analysis. DNA fragmentation assay also demonstrated that DNA ladder formation was observed at 24 hr after TB (20-30 μM) treatment to HL 60 cancer cells (Fig. 3C).

P21/Cip1 was the Key Regulator in the TB-Induced G0/G1 Arrest in the HL 60 cells

As shown in the figure 4, treatment of HL 60 cells with TB (5-20 μM) for 24 hr induced increased of p21/Cip1, and a decreased phospho-Rb protein expression in a dose-dependent manner. Interestingly, the p27/Kip1 was not significant changes in response to TB (Fig. 4). In order to determine whether the changes of the levels of cyclins and CDKs were also involved in the TB-induced G0/G1 arrest, we then examined cyclins and CDKs protein levels in response to TB treatment. TB at the concentrations of 1-20 μM did not induce any significant changes of the levels of

cyclin A, D1, D3 and proliferating cell nuclear antigen (PCNA). However, in the higher doses (40-60 μM)-treated groups, the cyclins A2, D1, and D3 protein levels were significantly down regulated in the HL 60 cells. Such results might be due to cellular toxicity occurred in the HL 60 cells treated with higher dose (40-60 μM) TB (data not shown).

TB-Induced G0/G1 Arrest Was through Inhibition of CDK2 and CDK4 Kinase Activity

It has been demonstrated that the progression of cell cycle activity is associated with coordinated successive activation of certain CDKs occurred late in the G1 phase and is instrumental in the transition from the G1 to the S phase [19, 20]. As described previously [21], the p21/Cip1 and p27/Kip1 were potent inhibitors of CDK2 and CDK4 which regulated the cells entering the restriction point in the G0/G1 phase of the cell cycle. Based on the flow cytometry analysis in the HL 60 cells (Fig. 3A), the time points at the 0, 15, 18 and 24 h after released from quiescence represents the G0/G1, S, G2/M and the sTBnd G0/G1 phases of the cell cycle, respectively. Accordingly, these time points were selected for Western blot analysis to examine the effects of TB on the expression of cell cycle regulatory proteins. We demonstrated that TB (20 μM) significantly suppressed CDK2 and CDK4 kinase activity (Fig. 4B) with concomitant increased p21/Cip1 protein expression (Fig. 4A). These results suggested that the G0/G1 arrest induced by TB was due to decreased of the CDK2 and CDK4 kinase activity mediated by an induction of p21/Cip1 expression.

TB Activated the Caspase 3, 8, and 9.

Since it has been demonstrated that the occurrence of apoptosis requires the activation of caspases [22], we investigated the involvement of caspase activation in the TB-induced apoptosis in the HL 60 cells by Western blot analyses. TB at higher dose (40-60 μM) activated the caspase 3 evidenced by a decreased in the protein level of procaspase 3 and degradation of the poly-ADP-ribose polymerase (PARP), the substrate for caspase 3 in both dose-dependent and time course studies (Fig. 5). To further elucidate the apoptotic pathways involved in the activation of caspase 3, we examined the changes of caspases 8 and 9 protein levels in the TB-treated HL 60 cells. Treatment of HL 60 cells with high dose (40-60 μM) TB activated caspase 9 evidenced by degradation of the procaspases 9 as well as the appearance of its cleavage product of caspase 8 (Fig. 5).

DICUSSION

TB has been used to treat superficial fungal infections and vaginal candidiasis. Successful treatment using TB in a case of middle aged women with opportunistic pulmonary aspergillosis had been reported [23]. Toxicity and teratogenicity studies in guinea-pigs and rats indicated that TB was well tolerated [24]. However, little is known about its use in oral or intravenous applications [25, 26]. The present study was undertaken to examine the anti-cancer effect of TB on human HL 60 cancer cells. Our *in vitro* studies demonstrated that TB at concentrations of 5-20 μM inhibited growth rate of HL 60 cells in a dose-dependent manner, but less significant in normal human fibroblasts. These results were due to an inhibitory effect of TB on the mechanisms for cell division in the subcultured cancer cells. Furthermore, when TB concentration was increased to 40-60 μM, apoptosis was observed in HL 60 cells. To our knowledge, this is the first demonstration that TB inhibits the growth of HL 60 cancer cells in an *in-vitro* experiment.

By flow cytometry analysis, we demonstrated that TB at lower concentrations (5-20 μM) induced G0/G1 cell cycle arrest of HL 60 cells. Cell cycle progression is regulated by successive, coordinated activation of CDKs [19, 20], whose activity is controlled by their association with a series of regulatory

subunits called cyclins and a group of CDK-inhibitory proteins designated CKIs [21]. Among these CKIs are two known as p21/Cip1 and p27/Kip1. The p21/Cip1 is induced by p53 tumor suppressor gene on DNA damage [27]. Transcriptional activation of p21/Cip1 associated and inactivated the CDKs which resulted in G0/G1 arrest [28, 29]. Keratinocyte from p21/Cip1-null mice are more tumorigenic than the wild type counterpart following expression of an activated ras allele [30] suggesting that p21/Cip1 is a candidate tumor suppressor gene. Our data demonstrated that p53 protein induction occurred in advance of p21/Cip1 suggesting that p53 might transcriptionally activated p21/Cip1 which in turn mediated TB-induced G0/G1 arrest in HL 60 cells.

p27/Kip1, a cell cycle regulatory protein, controls G1/S transition checkpoint by inhibiting the activity of different kinds of cyclin/CDK complexes *in vitro* and *in vivo,* including CDK2 and CDK4 complexes [31]. Homozygous deletion of p27/Kip1 alleles in mice results in enhanced growth, hyperplasia of various organs, and the spontaneous formation of pituitary tumors [32, 33]. Overexpression of p27/Kip1 leads to apoptotic cell death in mammalian cells suggesting a potential therapeutic approach aimed at elevating p27/Kip1 expression for treatment of human cancers [34]. In our study, low dose TB induced p21/Cip1 protein expression, suppressed the CDK2 and CDK4 kinase activity and induced G0/G1 arrest while there were no changes of cyclins or CDKs expression suggesting that p21/Cip1 was the main regulators of TB-induced cell cycle inhibition.

By flow cytometry and DNA fragmentation analyses, we also demonstrated that high dose TB (40-60 μM) caused apoptosis in HL 60 cells. The extrinsic, caspase 8/ FADD pathway and intrinsic, mitochondrial pathway are the two major signal pathways regulated apoptosis process. Activaton of bid by caspase 8 links the extrinsic to the intrinsic apoptotic pathway through mitochondrial damages [35]. It has been shown that clinically applied anticancer drugs such as cisplatin, topotecan, and gemcitabine induced apoptosis of solid tumor cells through caspase 8-dependent pathways [36]. Tumor resistance to cytotoxic drugs may occur through altered expression of caspase 8, upregulation of caspase 8 inhibitors or sequestration of caspase 8 [37]. Furthermore, hypoxia is known to be associated with interference to chemotherapy or radiotherapy and hypoxia-induced apoptosis mainly relies mitochondrial pathways [38]. Our results demonstrated that TB-induced apoptosis in colon cancer cell death was primarily through mitochondrial pathway which was not depended on caspase 8 and bid activation. These findings suggested that TB might be a useful salvage agent in the treatment of chemotherapy resistant colon cancer.

Previous studies suggested that Bax translocation to mitochondria was mediated by caspase activation [39, 40]. But our results demonstrated that pretreatment of pan-caspase inhibitor, Z-VAD-fmk failed to inhibit Bax translocation indicating that TBnzole-induced Bax translocation is caspase-independent. One of the major challenges in the cancer treatment is that many tumor cells carry mutations in key apoptotic genes such as p53, Bcl family proteins or those affecting caspase signaling. These defects resulted in failure of traditional chemotherapeutic treatments. Therefore, it is important to search for caspase-independent cell death mechanism in tumor [41]. It has been discovered that in response to apoptotic stimuli, mitochondria can also release caspase-independent cell death effector such as AIF [42]. AIF is a phylogenetically ancient mitochondrial intermembrane flavoprotein endowed with the unique capacity to induce caspase-independent peripheral chromatin condensation and large-scale DNA fragmentation when added to purified nuclei [43].

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Figure Legends

Figure 1. DNA fragmentation effects of TB on human HL 60 cancer cells. Human HL 60 cancer cells were treated with various types of antifungal agents (25 μM) for 24 hrs, cells were then collected and DNA gel electrophoresis performed.

Figure 2. Cell growth inhibitory effects of TB on human fibroblast and HL 60 cancer cells. (A) Human HL 60 cancer cells and (B) human normal fibroblasts were treated with various concentrations of TB (1-30 μM) for 1-5 days. Dose-dependent suppression of cell growth was observed in HL 60 cells and fibroblasts. Media with or without TB were changed everyday until cell counting. Three samples were analyzed in each group and the results were presented as means ± SE.

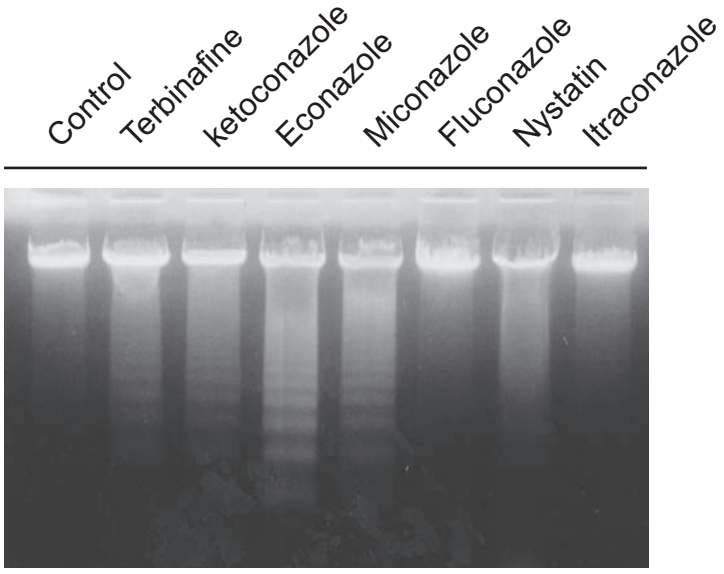
Figure 3. Effect of TB on cell cycle regulation of HL 60 cells. Time-dependent effects of (A) FACS analysis of DNA content was conducted after HL 60 cells were released from quiescence by incubation in culture media supplement with 10% FBS containing 0.05% DMSO (left) or

TB (25 μM in 0.05% DMSO, right panel). (B) Dose dependent response of TB on cell cycle regulation. HL 60 cells were quiescent and released by incubation in culture media supplement with 10% FBS and TB (1-60 μM) treatment for 24 h. Percentage of cells in sub-G1, G0/G1, S and G2/M phases of the cell cycle were determined using established CellFIT DNA analysis software. Three samples were analyzed in each group, and values were presented as means ± SE. (C) DNA fragmentation assay in HL 60 cells. Cells were treated with TB (1-30 μM) or DMSO (0.05%) as control and DNA fragmentation was examined 24 h later. M, molecular weight marker.

Figure 4. Effects of TB on the expression of G0/G1 phase regulatory proteins in HL 60 cells. (A) The cells were rendered quiescent by incubation for 24 h in the cultured media containing 0.04% FCS. After 15 h released from quiescence by incubation in culture media supplement with 10% FCS and 0.05% DMSO with or without TB (1-20 μM), the cells were harvested and protein extracts (100 μg per lane) were separate by SDS-PAGE. After electrophoresis, proteins were transferred onto Immobilion-P membranes, probed with proper dilution of specific antibodies, and then detected by using the NBT/BCIP system. Membrane was also probed with anti-GADPH antibody to correct for difference in protein loading. M, molecular weight marker. (B) Time course study of TB on the CDK2 and CDK4 kinase activity. The cells were rendered quiescent by incubation for 24 h in the cultured media containing 0.04% FCS. The cells then were released from quiescence by incubation in culture media supplement with 10% FCS and 0.05% DMSO with or without TB (20 μM) at the indicated time points, the cells were harvested and 200 μg protein per lane was used for the CDK2 and CDK4 kinase activity assay as described in the Materials and Methods.

Figure 5. Effect of TB on the activation of caspases proteins in HL 60 cells. The cells were rendered quiescent by incubation for 24 h in the cultured media containing 0.04% FCS. After 24 h released from quiescence by incubation in culture media supplement with 10% FCS and 0.05% DMSO with or without TB (5-60 μM), the cells were harvested and protein extracts (100 μg per lane) were separate by SDS-PAGE. After electrophoresis, proteins were transferred onto Immobilion-P membranes, probed with proper dilution of specific antibodies, and then detected by using the NBT/BCIP system. M, molecular weight marker.

Figure 1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Miconazole
Fluconazole
Nystatin
Itraconazole

Figure 2

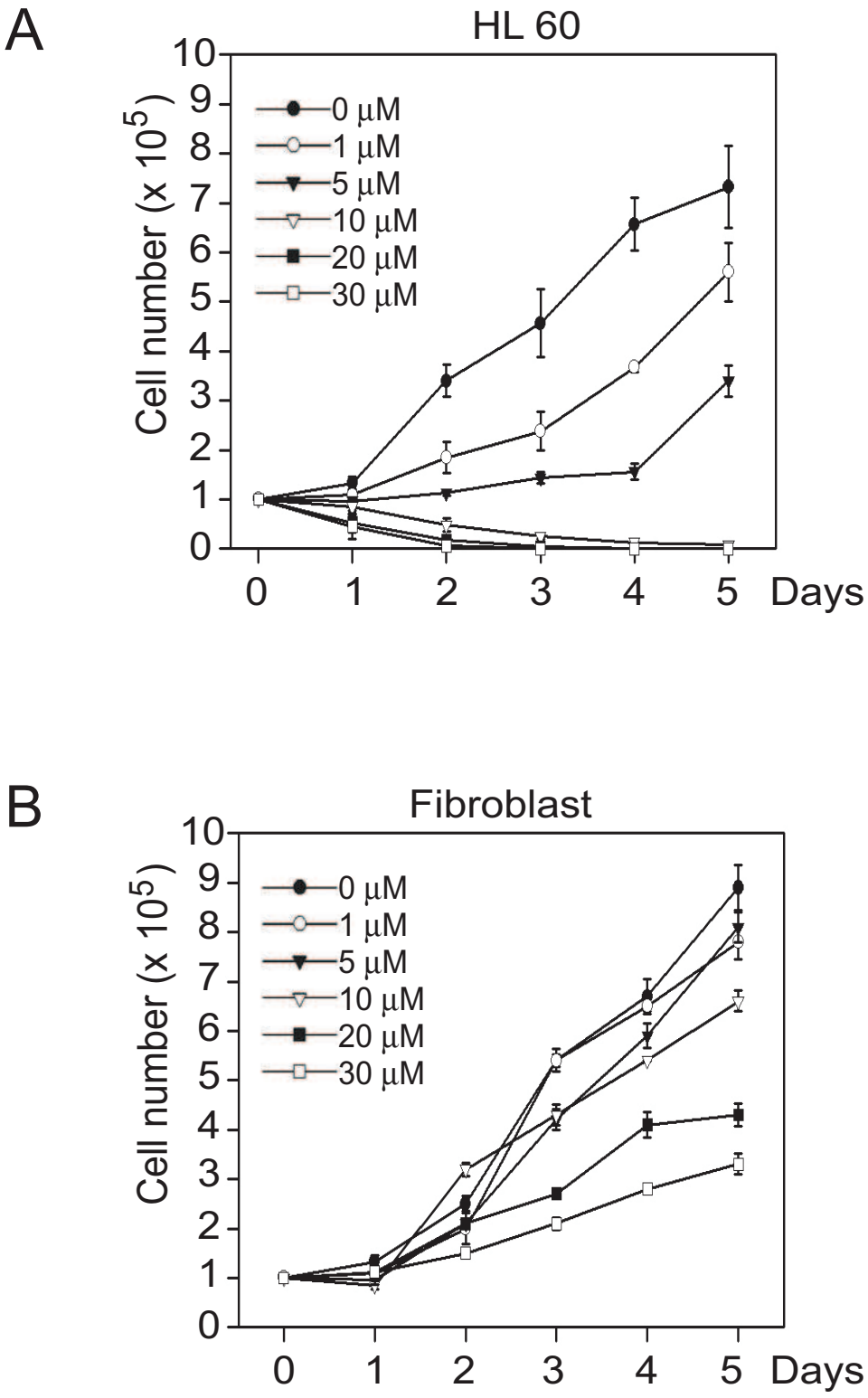

A
HL 60
Cell number (x 10^5)
0 μM
1 μM
5 μM
10 μM
20 μM
30 μM
Days
B
Fibroblast
Cell number (x 10^5)
0 μM
1 μM
5 μM
10 μM
20 μM
30 μM
Days

Figure 3

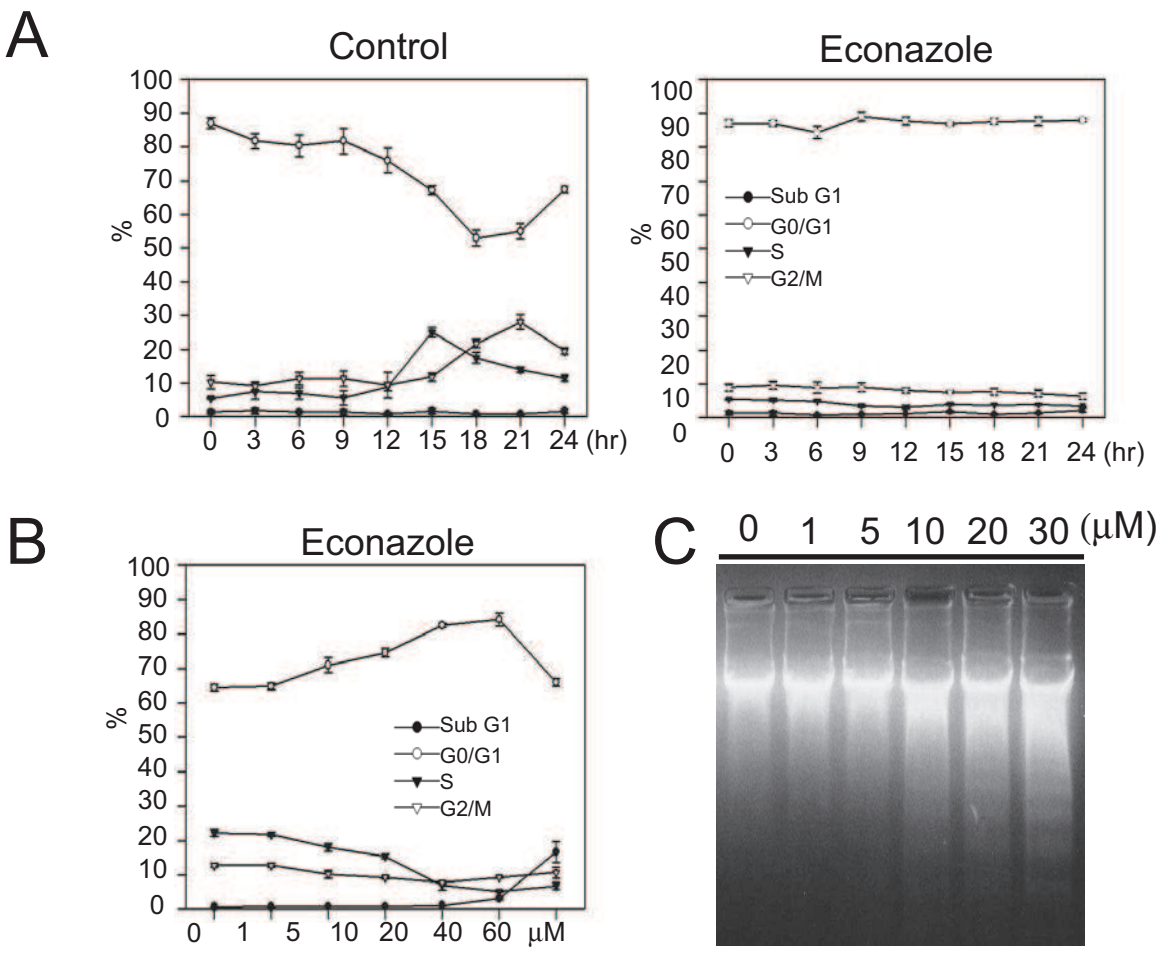

A
Control
Econazole
100
90
80
70
60
50
40
30
20
10
0
%
0 3 6 9 12 15 18 21 24 (hr)
Sub G1
G0/G1
S
G2/M
B
Econazole
0 1 5 10 20 40 60 μM
C
0 1 5 10 20 30 (μM)

Figure 4

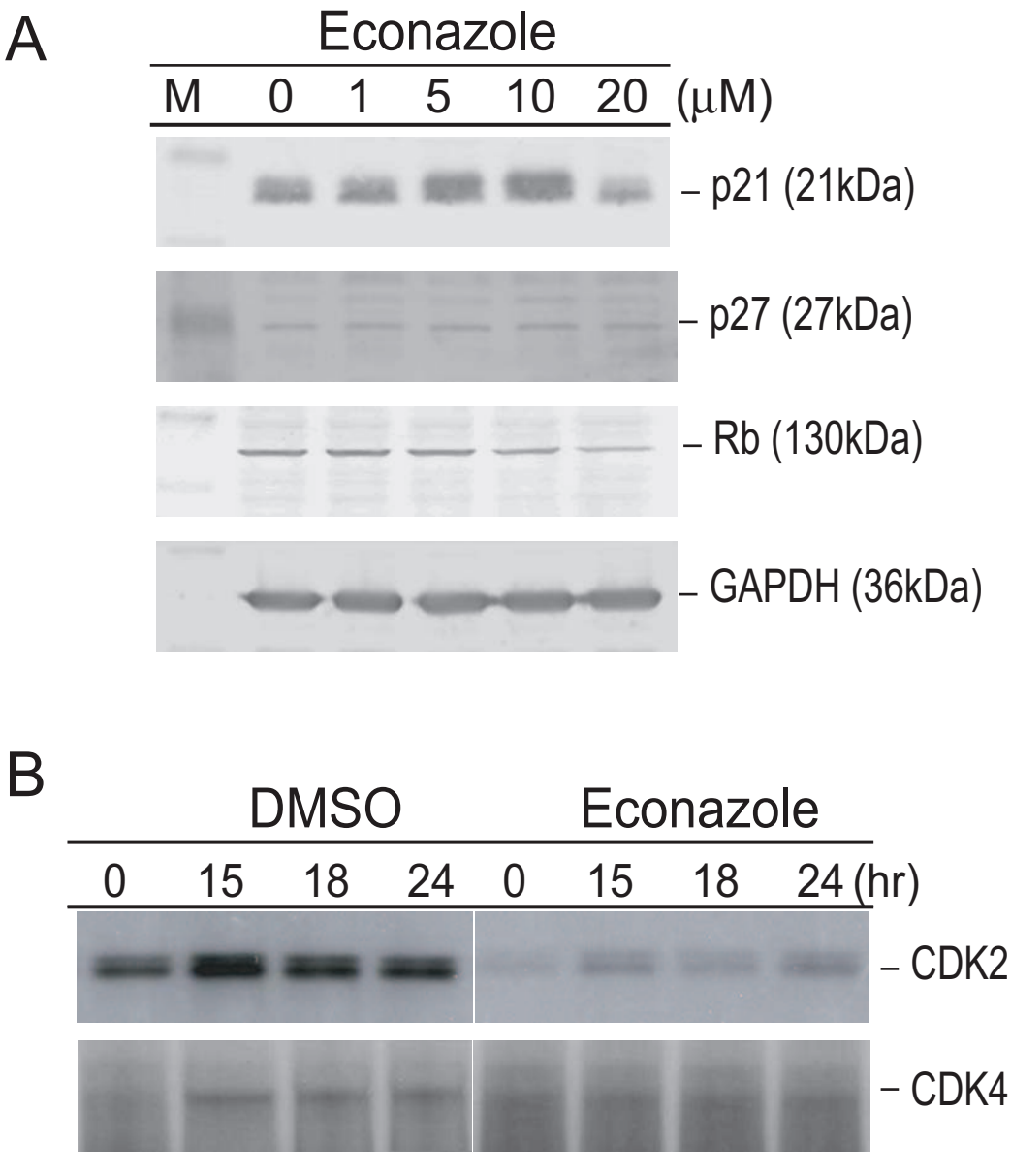
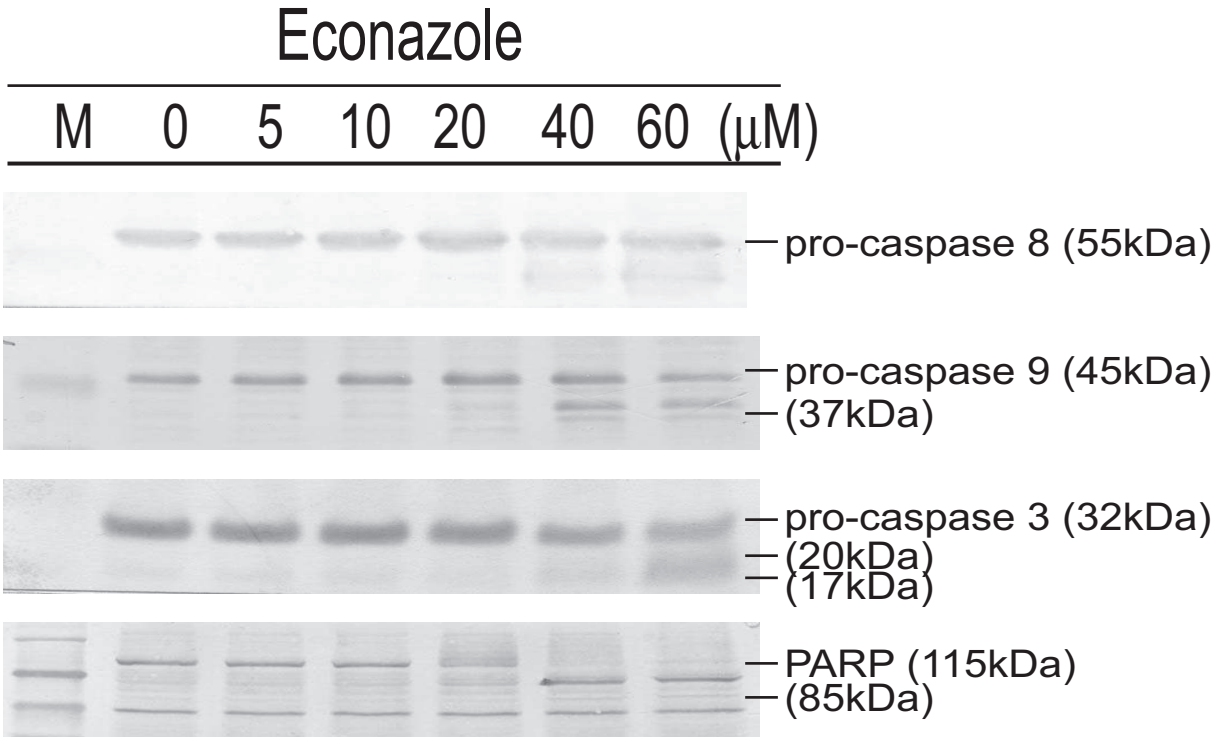

A
Econazole
M 0 1 5 10 20 (μM)
– p21 (21kDa)
– p27 (27kDa)
– Rb (130kDa)
– GAPDH (36kDa)
B
DMSO
Econazole
0 15 18 24 0 15 18 24 (hr)
– CDK2
– CDK4

Figure 5


Econazole
M 0 5 10 20 40 60 (μM)
pro-caspase 8 (55kDa)
pro-caspase 9 (45kDa)
(37kDa)
pro-caspase 3 (32kDa)
(20kDa)
(17kDa)
PARP (115kDa)
(85kDa)