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Abstract

Our previous studies have demonstrated that terbinafine (TB), a newly synthesized oral antimycotic drug, exerts anti-tumorigenesis and anti-angiogenesis activities. TB treatment caused cell cycle arrest at the G0/G1 phase through up-regulation of the p53 protein, which in turn caused an increase in p21 expression, and finally inhibited the cyclin-dependent kinase 4 (CDK4) activity in various cancer cells including colon and liver cancer cell lines. Moreover, administration of TB reduced the growth of tumors derived from human colon cancer cells in an *in vivo* setting. (Lee et al. 2003). In the human vascular endothelial cells, treatment with TB also caused growth inhibition through up-regulation of p53 and p21 protein, which in turn inhibited CDK2 kinase activity, and finally arrested cell cycle at the G0/G1 phase. Using tube formation and CAM assays, we further demonstrated that TB exerts anti-angiogenic activity (Ho et al. 2003). Taken together, we results strongly suggest the potential applications of TB in the treatment of human cancer. Although we are very happy with these exciting findings, several important issues still need to be further addressed before it can be applied for the clinical uses. Accordingly, the proposed grant application is aimed to apply the cellular and molecular biology techniques to further study the anti-cancer activity of TB in detail. In the present study, we found that TB dose-dependently inhibited adhesion and migration of HUVEC. The levels of phosphorylated ERK and FAK were downregulated in the TB-treated HUVEC. Pretreatment of HUVEC with TB prevented TB-induced inhibition of [3H]thymidine incorporation. Taken together, our data suggest that RAS, ERK and FAK might be involved in the TB-induced inhibition of angiogenesis. Using RT-PCR technique, we also demonstrated that the p21 mRNA levels were up-regulated in HUVEC after 6 hr treatment with TB, suggesting that TB-induced

increase of p21 protein is at the transcriptional level.

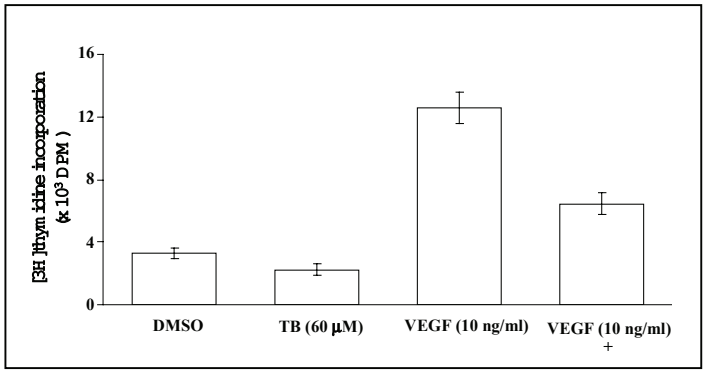
Introduction

TB is a newly synthesized oral antimycotic drug in the allylamines class: a fungicidal agent that inhibits ergosterol synthesis at the stage of squalene epoxidation. It shows a good safety profile and relatively few drug interactions. Recently, we have demonstrated that TB at a range of concentrations (0-120 μM) dose-dependently decreased cell number in various cultured human malignant cells. Flow cytometry analysis revealed that TB interrupts the cell cycle at the G0/G1 transition (Lee et al. 2004). The TB-induced cell cycle arrest in colon cancer cell line (COLO 205) occurred when the cyclin-dependent kinase (CDK) system was inhibited just as the levels of p53, p21 and p27 protein were augmented. In the TB-treated COLO 205, the binding between p53 protein and p53 consensus binding site in p21/Cip1 promoter DNA probe was increased. Pre-treatment of COLO 205 with p53-specific antisense oligodeoxynucleotide decreased the TB-induced elevations of p53 and p21/Cip1 protein, which in turn led to arrest the cell cycle at the G0/G1 phase. Moreover, in the p53 null cells, HL60, TB treatment did not induce cell cycle arrest. Taken together, these results suggest an involvement of the p53-associated signaling pathway in the TB-induced antiproliferation in COLO 205. We further examined whether administration of TB could affect the growth of tumors derived from human colon cancer cells in an *in vivo* setting. COLO 205 cells implanted subcutaneously in nude mice formed solid tumor; subsequent intraperitoneal injections of TB (50 mg/kg) led to obvious decline in tumor size of these tumors of up to 50-60%. In these tumors, increases in the p21, p27, and p53 protein and the occurrence of apoptosis were observed.

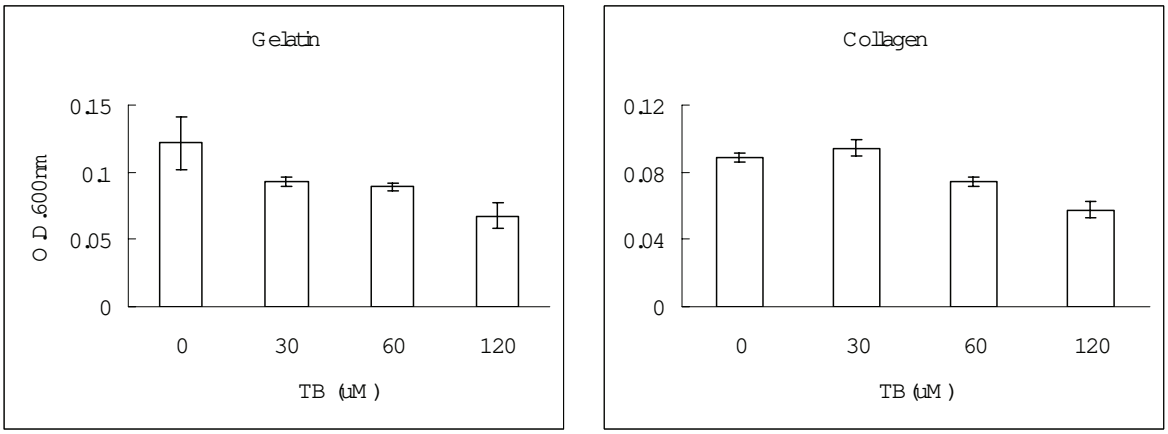
We also demonstrated that TB (0-120 μM) inhibited DNA synthesis and decreased cell number in cultured human vascular endothelial cells in a dose-dependent manner (Ho et al. 2004). TB was not cytotoxic at the concentrations used in the studies of cell growth and [3H]thymidine incorporation and this indicates that they may have an inhibitory effect on cell proliferation in the subcultured human vascular endothelial cells. Moreover, studies of [3H]thymidine incorporation revealed that treatment of the human vascular endothelial cells with TB decreased DNA synthesis and arrested the cells at the G0/G1 phase of the cell cycle. Western blot analysis demonstrated that the protein levels of cyclin A, but not cyclins B, D1, D3, and E, CDK2 and CDK4, decreased after TB treatment. The TB-induced cell cycle arrest in HUVEC occurred when the cyclin-dependent kinase 2 (CDK2) activity was inhibited just as the protein level of p21 was increased and cyclin A was decreased. Pretreatment of HUVEC with a p21 specific antisense oligonucleotide reversed the TB-induced inhibition of [3H]thymidine incorporation. Taken together, these results suggest an involvement of the p21-associated signaling pathway in the TB-induced antiproliferation in HUVEC. Capillary-like tube formation and chick embryo chorioallantoic membrane (CAM) assays further demonstrated the anti-angiogenic effect of TB. These findings demonstrate for the first time that TB can inhibit the angiogenesis.

Results

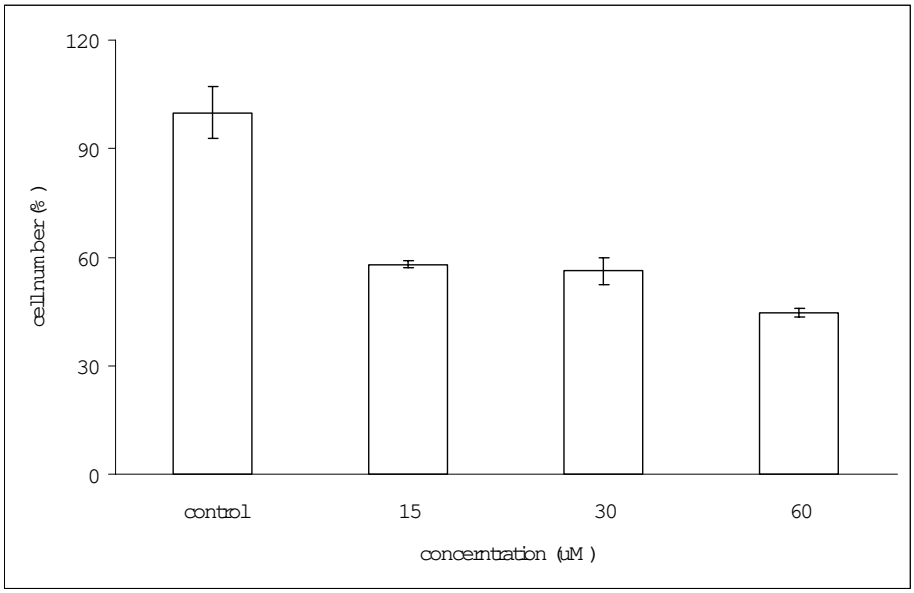
TB inhibits VEGF-induced [3H]thymidine incorporation in HUVEC



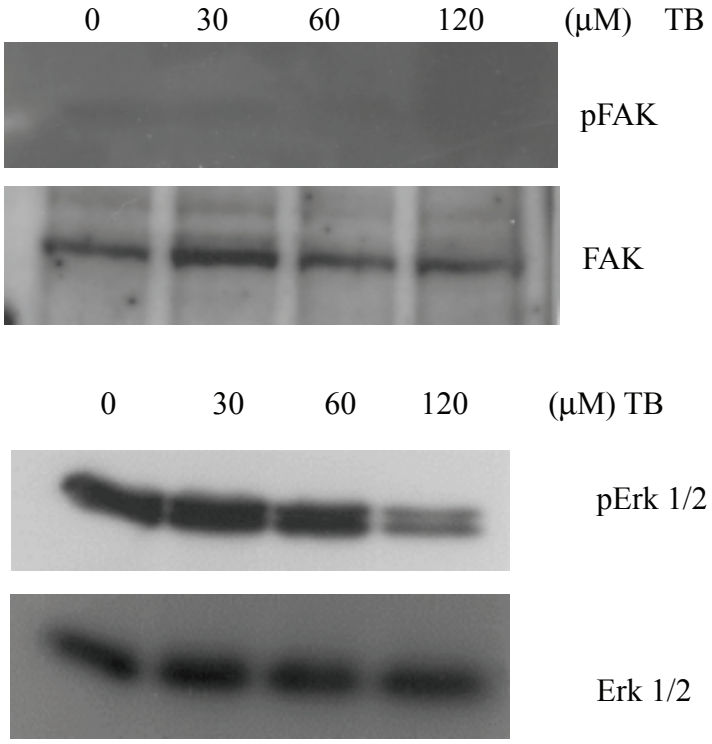
TB dose-dependently inhibits HUVEC adhesion in gelatin and collagen-coating plates



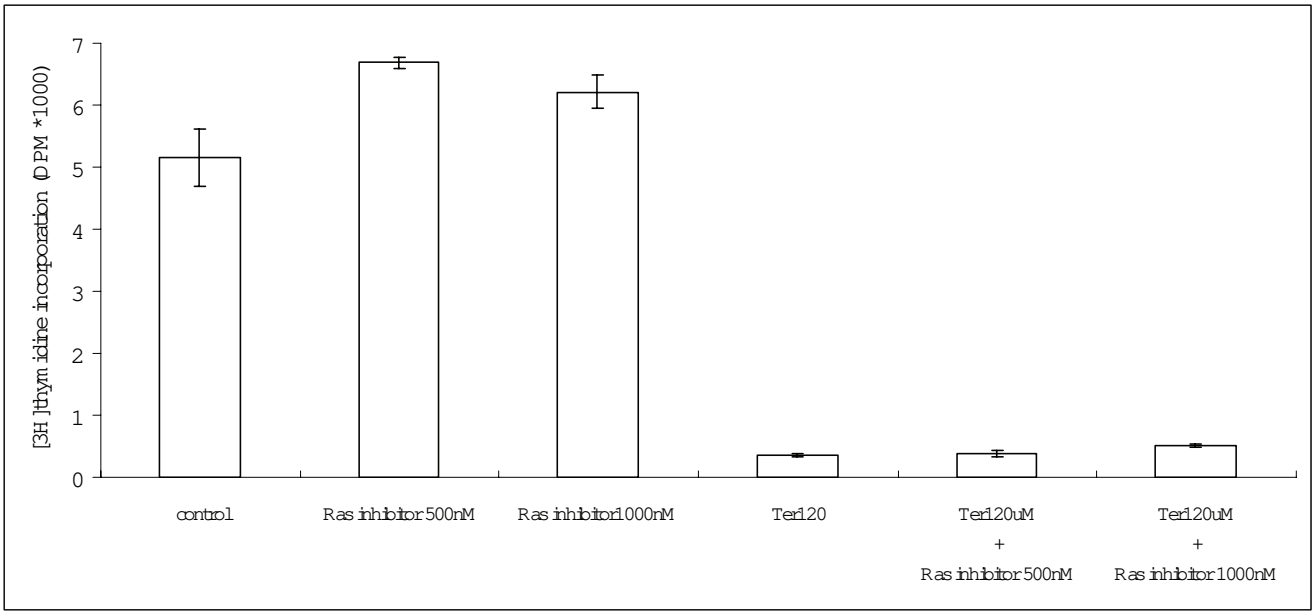
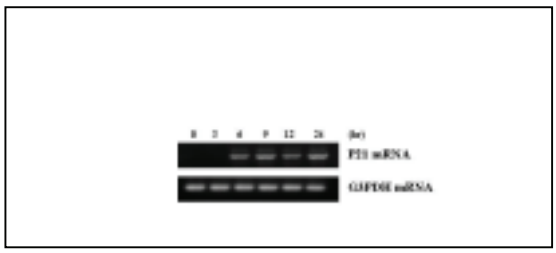
TB dose-dependently inhibits HUVEC migration



TB dose-dependently inhibits phosporylations of FAK and ERK



RAS inhibitor reverses TB-induced inhibition of [3H]thymidine incorporation



The levels of p21 mRNA are increased at 6 hr after TB treatment