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Research paper

Thymoquinone inhibits microtubule polymerization by tubulin binding and causes mitotic arrest following apoptosis in A549 cells

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ABSTRACT

Microtubule-Targeting agents (MTA) are indispensable for cancer therapeutics. We here report thymoquinone (TQ) as a new MTA that already has been appreciated for its anticancer effects. TQ induced G_2/M cell cycle arrest in human non-small lung epithelial cells (A549) and majority of arrested cells were in mitosis. TQ depolymerized the microtubule (MT) network and disrupted mitotic spindle organization of A549 cells. MT depolymerization by TQ was followed by apoptosis and subsequent loss in cell viability (IC_{50} value of ~10 μM). Interestingly, TQ didn't affect the MT network of normal HUVEC cells at and below the IC_{50} concentration for A549 cells. TQ also inhibited tubulin polymerization in *cell-free system* with an IC_{50} of 27 μM and bound to tubulin heterodimers at a single site with a dissociation constant of 1.19 μM at 25 °C. Binding of TQ to tubulin quenched the tryptophan fluorescence of protein in a time-dependent manner. The TQ–tubulin binding kinetics was biphasic in nature and equilibrated in 30 min. TQ competed with colchicine for tubulin binding with a K_i of 1.9 μM as determined by modified Dixon plot analysis, this suggests TQ may bind tubulin at or near the colchicine binding site and *in silico* modeling study supported that. Our results establish a novel antimitotic mechanism of TQ by its direct binding to tubulin-MT network in A549 cells.

1. Introduction

Microtubules (MT) are dynamic polymers of α and β tubulin heterodimers regulate versatile cellular functions including mitosis, cell motility, maintenance of cell shape and structure, cell signaling, and organelle transport [1–4]. MT plays a critical role in the formation and functioning of this spindle apparatus and improper arrangement of it could be fatal for cell division [5]. MT network has emerged as one of the most effective targets for cancer therapeutics because of its crucial roles in cellular physiology [6–15]. There are two distinct types of MTAs interact either with tubulin dimers or MT polymer [7]. MT depolymerizer or destabilizers; preferably interact with α/β-tubulin heterodimers and inhibit MT polymerization like vinblastine, nocodazole, combretastatin, estramustine, colchicine etc. MT stabilizers; preferentially bind to existing MT polymers and alter their dynamic instability and stabilize them like paclitaxel, epothilone etc. Structurally dissimilar microtubule binding agents mainly bind at one of the three sites: the vinblastine site, the colchicine site or the taxol site. Despite of having tremendous therapeutic success the dose limiting cytotoxicity, neurological and hematological side-effects affected the applicability of MTAs in cancer therapy. In addition to these, the expression of multidrug–drug resistance proteins and the tubulin isotype specificity have made many cancer cells resistant to the clinically successful antimitotic agents [15]. Therefore, it is urgent to find more structurally-diverse MTAs and clinically potential for future antitumor therapy.

Natural compounds have always been the richest resource for anticancer agents. Thymoquinone (TQ) (Fig. 1A for structure), a naturally occurring quinone (2-isopropyl-5-methylbenzo-1,4-quinone) is the predominant active component (30%–48%) of black cumin (*Nigella sativa*) seed oil [16,17]. Therapeutically, seed oil has been used against hypertension, lung disease, arthritis, and hypercholesterolemia for more than two thousand years [17,18]. TQ

Abbreviations: TQ, thymoqionone; ROS, reactive oxygen species; PIPES, 1,4-piperazinediethanesulfonic acid; EGTA, ethylene bis(oxyethylenenitrilo) tetra-acetic acid; DMSO, dimethyl sulfoxide; GTP, guanosine 5′-triphosphate; DAPI, 4′,6-diamidino-2-phenylindole; PI, propidium iodide; MTT, (3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide).

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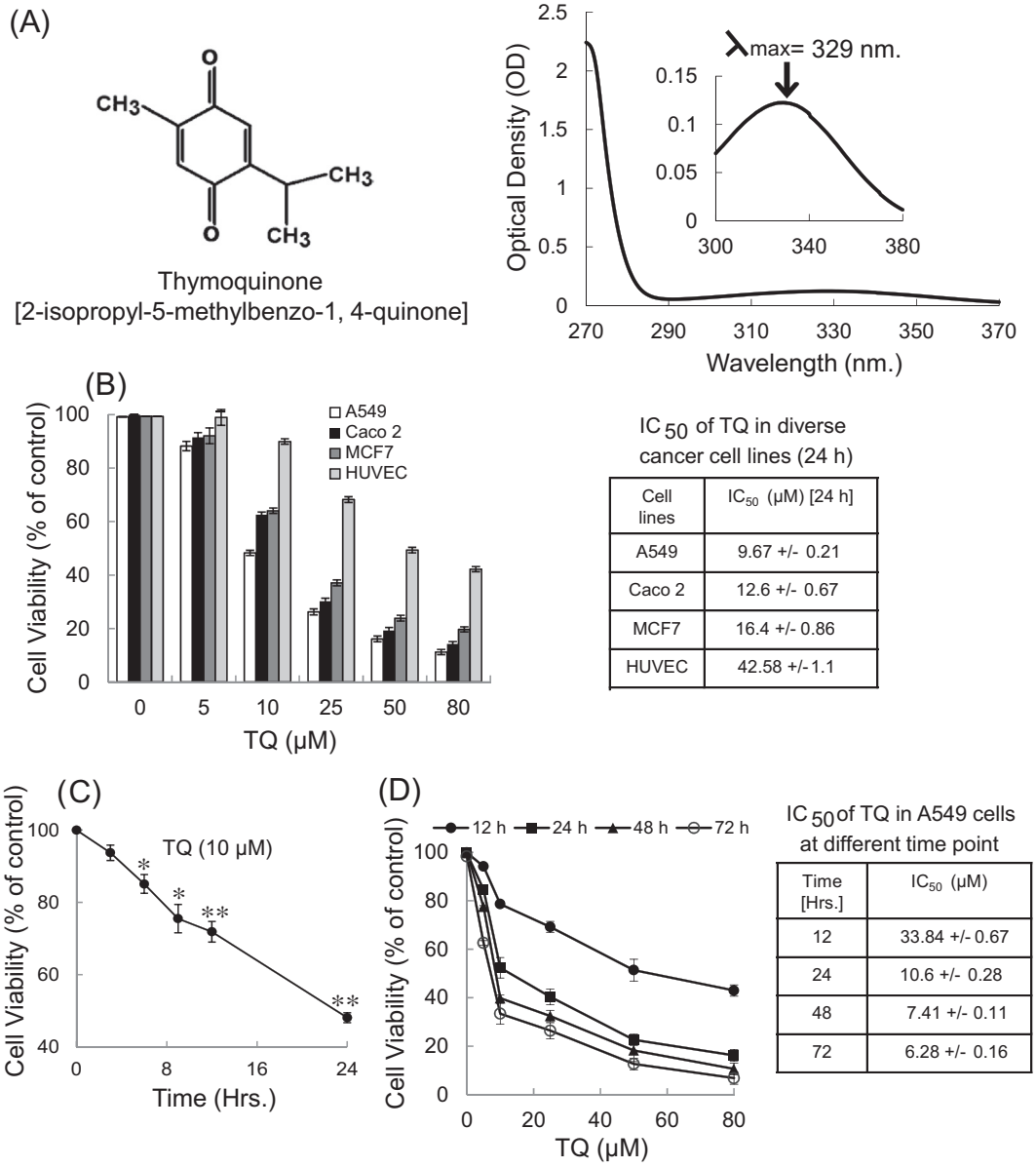

Fig. 1. Effect of TQ cell viability of tumor cells. (A) Chemical structure of TQ thymoquinone [2-isopropyl-5-methylbenzo-1,4-quinone] and the absorption spectrum of 50 μM TQ [inset showing the absorption maxima pick at 329 nm]. (B) IC_{50} concentrations of TQ in different cell lines was measured by MTT assay for 24 h in the presence of various concentrations (0–80 μM) of TQ. (C) The reduced viability of A549 cells was measures in the presence of 10 μM TQ up to 24 h. (D) The reduced viability of A549 cells was measured by MTT assay for different time points (0–72 h) after TQ (0–80 μM) treatment. The data represent as mean ± SEM (*$P < 0.05$ and **$P < 0.01$ compared to control, $n = 3$).

has antioxidant and anti-inflammatory properties [19,20]. TQ effects have been examined against intrinsic tumor pathophysiology; it inhibits the proliferation of different types of cancer cells in culture [16,21]. TQ treatment induced both G_1 and G_2/M cell cycle arrest and altered cyclin dependent kinase activity in specific cancer cell types [21–28], it induces apoptosis by enhancing the Bax/BCl-2 ratio and NFκB down regulation in cancer cells [29]. TQ is also reported to exert its anti-cancer effect by intracellular ROS generation [30].

The purpose of this entire study was to determine a novel antimitotic mechanism of TQ by tubulin binding. In our study, we found that TQ directly interact with tubulin and binds at its colchicine binding site. TQ strongly inhibited *in vitro* tubulin polymerization and induced depolymerization of cellular microtubule and spindle abnormality in A549 cells. Such disruption of microtubule dynamics further led to cell cycle arrest in G_2/M phase which proceeded to apoptosis and reduced cell viability in A549 cells. Interestingly, TQ was more effective in cancer cells versus normal human cells. However, in depth mechanistic evaluation and clinical correlations are required to consider TQ as a potential future MTA against several types of cancers.

2. Materials and methods

2.1. Reagents and antibodies

Rhodamine tubulin was purchased from Cytoskeleton, Inc., USA. TQ, DAPI, anti-α-tubulin antibody (mouse monoclonal), anti-p53 antibody (mouse monoclonal), anti-Bax antibody (mouse monoclonal), anti-Bcl-2 (mouse monoclonal), Rhodamine-labeled anti-mouse secondary antibody, Guanosine 5′-triphosphate (GTP), PIPES, $MgCl_2$, zVAD-fmk, EGTA were purchased from SIGMA, USA.