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TW01001, a novel piperazinedione compound, induces mitotic arrest and autophagy in non-small cell lung cancer A549 cells

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ABSTRACT

Here, we report that TW01001, a novel piperazinedione compound, could be a new mitotic inhibitor for the treatment of non-small cell lung cancer by the following observations in A549 cells: (1) induction of cells to accumulate at G2/M phase, which ultimately led to cell apoptotic death, (2) accumulation of p53 and inhibition of survival signalings, and (3) induction of p53-independent autophagy. Taken together, our data suggested that TW01001 induces autophagy-p53-signaling pathway to cause mitotic arrest and cell growth inhibition in A549 cells and provides the framework for further development as a novel therapeutic agent for lung cancer treatment.

1. Introduction

Anti-mitotic therapies have been widely used for decades in patients with a variety of malignancies, including hematological malignancies, breast cancer, ovarian cancer, and non-small-cell lung carcinoma [1,2]. Although anti-mitotic agents are sometimes considered "old fashioned" compared to current anticancer approaches [1,2], they still show impressive success in patients and remain scientifically interesting [3]. New anti-mitotic compounds, especially those with novel chemical structures, are always attractive in the field of anticancer drug development, as they might show promising anti-tumor activities in preclinical model systems [4–8].

Cyclin-dependent kinase 1 (Cdk1; also called p34cdc2) complexes with cyclin B1 to form the "mitosis promoting factor," which is expressed predominantly during the G2/M phase of the cell cycle. Progression from G2 to M phase is driven by activation of the Cdk1/cyclin B1 complex, which is controlled by phosphorylation at different sites of Cdk1 [9,10]. Induction of aberrant mitosis in tumor cells leads to mitotic arrest, which is frequently followed by apoptotic cell death [11–13]. Apoptosis, which is the best-known form of programmed cell death, involves the activation of catabolic enzymes to trigger multi-step pathways lead to characteristic biochemical and morphological changes [14,15]. Another self-destructive process, autophagy, is used to eliminate cellular proteins and cytoplasmic organelles [16–18]. The relationship between apoptosis and autophagy is complex and highly context-dependent; in several scenarios, autophagy constitutes a stress adaptation that protects the cell [19], whereas in other cellular settings, it leads to autophagic cell death [20]. Autophagy is believed to play an important role in regulating cancer development, malignant progression, and the response of tumor cells to anticancer treatment [20–22].

We recently identified TW01001 [sodium 3-((6-((Z)-((Z)-5-benzylidene-3,6-dioxopiperazin-2-ylidene)methyl)pyridin-3-yloxy)methyl)benzoate; structure shown in Fig. 1A] as a potent growth inhibitor and inducer of mitotic arrest in the A549 human non-small cell lung cancer cell line, focusing on this cell line due to the poor prognosis and lack of effective therapies in lung carcinoma patients. Here, we characterized the effects and action mechanism of TW01001 in A549 cells. We found that TW01001 is a potent mitotic inhibitor that induces autophagy in A549 cells. Furthermore, inhibition of autophagy by chemical or siRNA-mediated knockdown protected the cells against TW01001-induced apoptotic cell death,

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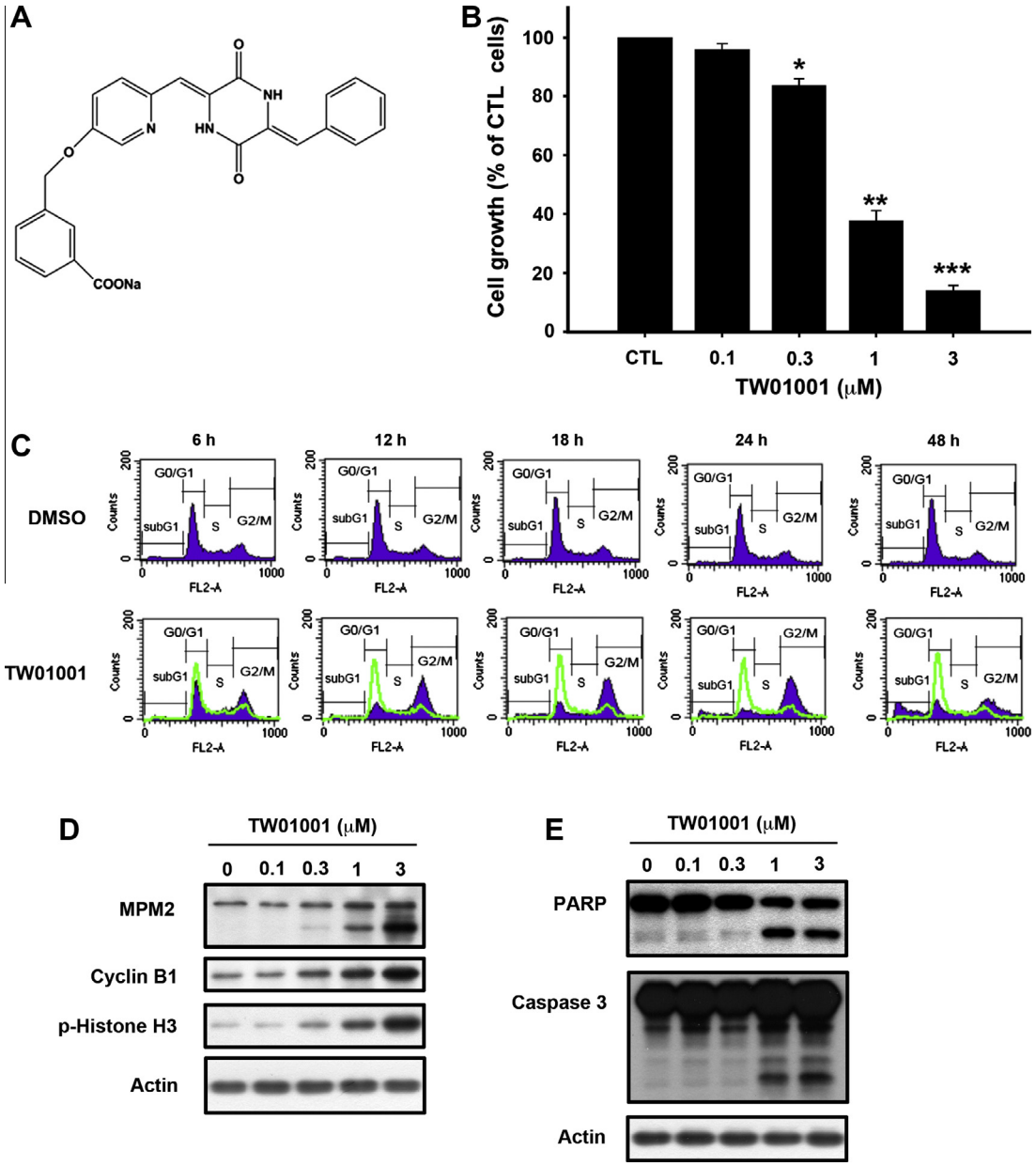

Fig. 1. Chemical structure of TW01001 and its effect on cell viability and cell cycle distribution. (A) The chemical structure of TW01001. (B) Concentration-dependent effect of TW01001 on cell growth. A549 cells were incubated with or without the indicated concentrations of TW01001 for 48 h, and cell growth was evaluated by SRB assay. Data are expressed as the mean ± S.D. of at least three independent experiments. Symbols: *, $P < 0.05$; **, $P < 0.01$; and ***, $P < 0.001$ compared with the control group. (C) Time-dependent effect of TW01001 on cell cycle progression in A549 cells. Cells were treated with DMSO or TW01001 (1 μM) for the indicated times and the cell cycle distribution was analyzed by flow cytometry. (D) Concentration-dependent effect of TW01001 on the induction of mitotic arrest. Cells were treated with the indicated concentrations of TW01001 for 24 h and cell lysates were subjected to immunoblotting using the indicated antibodies. (E) Effect of TW01001 on apoptosis in A549 cells. Cells were treated with the indicated concentrations of TW01001 for 48 h, and whole-cell extracts were subjected to Western blot analysis using anti-caspase-3, anti-PARP and anti-β-actin antibodies.

suggesting that TW01001-induced autophagy plays an essential role in this apoptotic signaling. In sum, we herein demonstrate for the first time that TW01001 induces mitotic arrest and apoptosis through the induction of autophagy. Thus, targeting the autophagic pathway could provide a potential strategy for treating non-small cell lung cancer.

2. Materials and methods

2.1. Cell lines and reagents

A549 and H1299 cells were obtained from the American Type Culture Collection (ATCC) (Manassas, VA, USA). Cells were maintained in 10% fetal bovine serum (FBS)-supplemented RPMI 1640 medium (GIBCO, Grand Island, NY, USA) and 1% penicillin–streptomycin (GIBCO) at 37 °C in a humidified incubator containing 5% CO_2. TW01001, sodium 3-((6-((Z)-((Z)-5-benzylidene-3,6-dioxopiperazin-2-ylidene)methyl)pyridin-3-yloxy)methyl)benzoate (Fig. 1A), was obtained from Professor Hui-Po Wang (College of Pharmacy, Taipei Medical University, Taiwan). It is a potential lead compound from a series of cyclic azatyrosinamide derivatives structurally modified from antioncogenic azatyrosine [23]. Azatyrosine demonstrated poor intracellular bioavailability, it was used as a template to design azatyrosinamides which had improved cell penetration and significant cytotoxicity [23]. Rationale behind the design on the novel azatyrosine-containing piperazinediones is that the antitumoral activities might be increased as a consequence of increased intracellular bioavailability due to their high lipophilicity. Antibodies against various proteins were obtained from the following sources: PARP (Poly-ADP-ribose polymerase), cyclin B1, Bcl-2, Bcl-xL, Bax, p21, p62, puma, Hec1, Nek2, and anti-mouse and anti-rabbit IgGs were obtained from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA). Phospho-p53 (Ser15), Bcl-2 (Ser70), phospho-Akt (Ser473), phospho-Stat3 (Tyr705), Stat3, Akt, phospho-p44/42 MAPK (1/2 Erk) (Thr202/Tyr204),