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Thiamet-G-mediated inhibition of O-GlcNAcase sensitizes human leukemia cells to microtubule-stabilizing agent paclitaxel

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ABSTRACT

Although the microtubule-stabilizing agent paclitaxel has been widely used for treatment of several cancer types, particularly for the malignancies of epithelia origin, it only shows limited efficacy on hematological malignancies. Emerging roles of O-GlcNAcylation modification of proteins in various cancer types have implicated the key enzymes catalyzing this reversible modification as targets for cancer therapy. Here, we show that the highly selective O-GlcNAcase (OGA) inhibitor thiamet-G significantly sensitized human leukemia cell lines to paclitaxel, with an approximate 10-fold leftward shift of IC_{50}. Knockdown of OGA by siRNAs or inhibition of OGA by thiamet-G did not influence the cell viability. Furthermore, we demonstrated that thiamet-G binds to OGA in competition with 4-methylumbelliferyl N-acetyl-β-D-glucosaminide dehydrate, an analogue of O-GlcNAc UDP, thereby suppressing the activity of OGA. Importantly, inhibition of OGA by thiamet-G decreased the phosphorylation of microtubule-associated protein Tau and caused alterations of microtubule network in cells. It is noteworthy that paclitaxel combined with thiamet-G resulted in more profound perturbations on microtubule stability than did either one alone, which may implicate the underlying mechanism of thiamet-G-mediated sensitization of leukemia cells to paclitaxel. These findings thus suggest that a regimen of paclitaxel combined with OGA inhibitor might be more effective for the treatment of human leukemia.

1. Introduction

The antineoplastic activity of paclitaxel has been demonstrated for several types of cancer, particularly for the malignancies of epithelia origin, for instance breast and lung cancer [1,2]. Paclitaxel acts as a microtubule-stabilizing agent that interferes the microtubule depolymerization, resulting in cell cycle arrest at G2/M transition stage [3]. In contrast to those cancer types that are sensitive to paclitaxel, acute leukemia only responded modestly to paclitaxel treatment [4]. Thus, it has been suggested that paclitaxel might be administered in combination with other agents for the chemotherapy for hematologic malignancies [4].

O-GlcNAcylation refers to the posttranslational modification of O-linkage of N-acetyl-glucosamine moieties to serine and threonine residues on cytoplasmic and nuclear proteins [5]. The O-GlcNAc transferase (OGT) and the O-GlcNAcase (OGA), which transfers GlcNAc to and removes GlcNAc from proteins, respectively, are the key enzymes in the reversible reaction [6]. The roles of O-GlcNAcylation modification of proteins have been implicated in multiple aspects of cell physiology and pathobiology [7], such as nutrition metabolism [8], signaling in cardiovascular system [9,10], neurodegenerative disorders [11], and cancer [12,13]. It is noteworthy that O-GlcNAcylation is dynamically regulated [14,15]. In Alzheimer's disease, the reciprocal relationship between O-GlcNAcylation and phosphorylation of tau has been taken advantage for drug design [16,17]. Tau is a microtubule-associated protein and hyperphosphorylation of tau is a pathologic feature of Alzheimer's disease. The newly emerged O-GlcNAcase selective inhibitor thiamet-G has been shown to reduce the phosphorylation of tau at pathologically relevant serine and threonine sites in neuron cells [16], suggesting its potential value in the therapy for tau hyperphosphorylation-associated neurodegenerative disorders.

In light of that both paclitaxel and thiamet-G exert effects on microtubule organization directly or indirectly, we hypothesized that paclitaxel in combination with thiamet-G may give rise to more profound cytotoxicity to human leukemia cells than the

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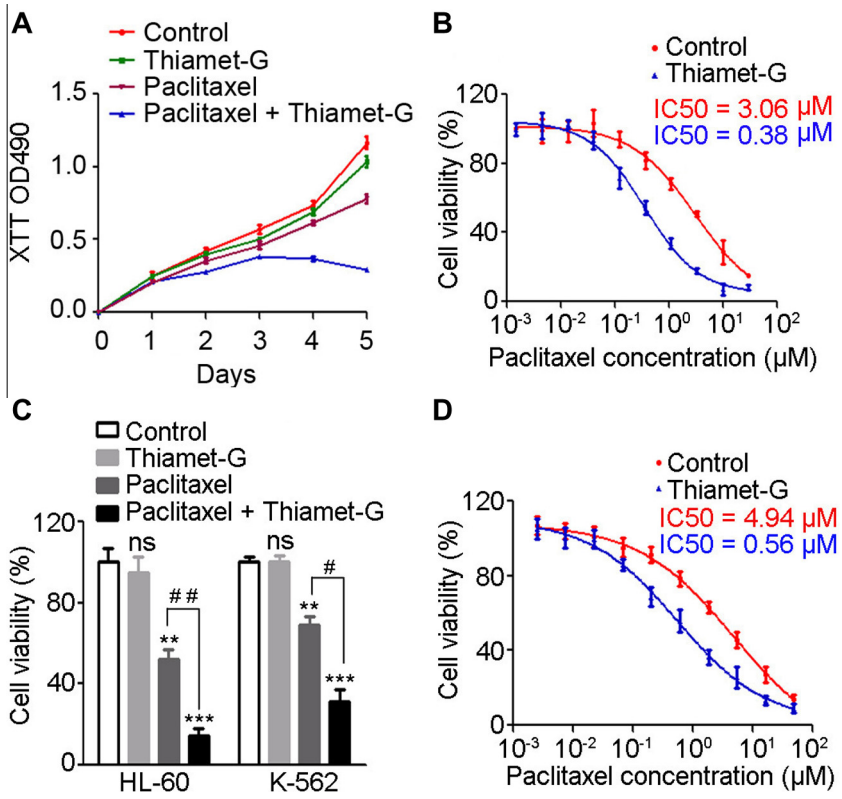

Fig. 1. Inhibition of O-GlcNAcase sensitizes leukemia cells to paclitaxel treatment. (A) Jurkat cells were treated with DMSO, paclitaxel (10 μM), Thiamet-G (10 μM), or paclitaxel (10 μM) in combination with Thiamet-G (1 μM) for the indicated time, and cell viability was analyzed by XTT assay. (B) Jurkat cells were treated with serially diluted paclitaxel in the presence or absence of Thiamet-G (1 μM), and cell viability was determined by XTT assay. (C) HL-60 cells and K-562 cells were treated with DMSO, paclitaxel (10 μM), Thiamet-G (10 μM), or paclitaxel (10 μM) in combination with Thiamet-G (1 μM) for 3 days, and cell viability was analyzed by XTT assay. (D) HL-60 cells were treated with serially diluted paclitaxel in the presence or absence of Thiamet-G (1 μM), and cell viability was determined by XTT assay. $^{**}P < 0.01$ versus control; $^{***}P < 0.001$ versus control; ns, not significant; $^{\#}P < 0.05$ versus paclitaxel; $^{\#\#}P < 0.01$ versus paclitaxel.

paclitaxel alone. Here, our results support the hypothesis by showing that the OGA-selective inhibitor thiamet-G significantly sensitized human leukemia cells to paclitaxel-mediated cytotoxicity. The underlying mechanism of sensitization could be due to synergistic effects of paclitaxel and thiamet-G on the perturbation of microtubule network.

2. Materials and methods

2.1. Reagents

Thiamet-G and paclitaxel were purchased from Selleck (TX, USA) and dissolved in DMSO. 4-Methylumbelliferyl N-acetyl-β-D-glucosaminide dehydrate was obtained from Sigma (MO, USA). Purified O-GlcNAcase was purchased from Origene (MD, USA). siRNAs targeting OGA and luciferase (control) were synthesized by Invitrogen (Beijing, China). 2,3-Bis(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide (XTT) was from Promega (WI, USA). Antibodies against O-GlcNAc (CTD110.6), Tau, and phosphorylated Tau were from Santa Cruz Biotech (CA, USA). Antibodies against β-actin and α-tubulin (DM1A) were obtained from Abcam (MA, USA). Horseradish peroxidase-conjugated secondary antibody and FITC-conjugated secondary antibody were obtained from Jackson ImmunoResearch Laboratories (PA, USA).

2.2. Cell culture and treatment

The human leukemia cell lines Jurkat E6-1, HL-60, and K-562 were purchased from the American Type Culture Collection (ATCC), maintained in RPMI1640 medium supplemented with 10% FBS, and incubated at 37 °C, 5% CO_2. siRNAs were transfected into cells by using lipofectamine 2000 (Invitrogen) according to the manufacture's protocol. For microtubule stability analysis, cells treated with DMSO, paclitaxel, thiamet-G, or paclitaxel combined with thiamet-G were incubated on ice for 20 min.

2.3. Enzymatic assay

All enzymatic assays were performed in triplicate at 37 °C using 4-methylumbelliferyl N-acetyl-β-D-glucosaminide dehydrate as substrate. 1 nM of purified OGA was incubated with the compounds for 5 min, and then 0.2 mM of the substrate was added. The liberation of 4-methylumbellifery was monitored by kinetic reading at excitation/emission 355/460 nm using a Tecan M200 plate in a mode of 60 s/cycle and 15 cycles in total.

2.4. Determination of inhibition mode of OGA

Purified OGA was incubated with thiamet-G for 5 min, and then different concentration of substrate was added to initiate the reaction. The reaction was monitored by kinetic reading at excitation/emission 355/460 nm. The inhibition mode was analyzed by Lineweaver–Burk plots.

2.5. Proliferation assay

Jurkat cells were seeded at 6000 cells/well in a 96-well plate, and 12 h later, cells were treated with compounds for the indicated