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Original Articles

Inhibition of Aurora kinases induces apoptosis and autophagy via AURKB/p70S6K/RPL15 axis in human leukemia cells

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ABSTRACT

Leukemia is a common malignancy of blood cells with poor prognosis in many patients. Aurora kinases, a family of serine/threonine kinases, play a key role in regulating cell division and mitosis and are linked to tumorigenesis, metastasis, and poor prognosis in many human cancers including leukemia and lymphoma. Danusertib (Danu) is a pan-inhibitor of Aurora kinases with few data available in leukemia therapy. This study aimed to identify new molecular targets for Aurora kinase inhibition in human leukemia cells using quantitative proteomic analysis followed by verification experiments. There were at least 2932 proteins responding to Danu treatment, including AURKB, p70S6K, and RPL15, and 603 functional proteins and 245 canonical signaling pathways were involved in regulating cell proliferation, metabolism, apoptosis, and autophagy. The proteomic data suggested that Danu-regulated RPL15 signaling might contribute to the cancer cell killing effect. Our verification experiments confirmed that Danu negatively regulated AURKB/p70S6K/RPL15 axis with the involvement of PI3K/Akt/mTOR, AMPK, and p38 MAPK signaling pathways, leading to the induction of apoptosis and autophagy in human leukemia cells. Further studies are warranted to verify the feasibility via targeting AURKB/p70S6K/RPL15 axis for leukemia therapy.

Introduction

Cancer is a major disease burden globally that continues to increase largely due to aging, population growth, and an increasing adoption of cancer-causing behaviors. There were 14.1 million new cancer cases, 8.2 million cancer deaths and 32.6 million people living with cancer (within 5 years of diagnosis) in 2012 worldwide [1]. Leukemia is a common malignancy of blood cells emerging from different cell types, and it mainly includes acute lymphoblastic leukemia, acute myelogenous leukemia (AML), chronic lymphocytic leukemia, and chronic myelogenous leukemia (CML). Leukemia is the 11th most common cancer worldwide, with around 352,000 new cases diagnosed in 2012 (2% of the total) [1]. In 2015, more than 54,000 American people were diagnosed with leukemia, at least 327,000 people lived with leukemia, and more than 24,400 people died from leukemia in the US [2]. AML, the most prevalent acute leukemia in adults, still remains the most difficult malignant cancer to cure, without significantly changed mainstays of treatment in the past two decades [3]. On the other hand, there is a growing interest in the discovery and identification of new therapeutic targets to inhibit cancer development and progression; however, the clinical outcome of cancer therapy is disappointing with low overall response and survival rate due to drug resistance and disease reoccurrence. Thus, there is an urgent need to discover and develop new therapies for leukemia therapy with more potent therapeutic efficacy and fewer side effects through targeting novel molecular targets.

Aurora kinases represent a family of serine/threonine kinases that play a key role in regulation of cell division and mitosis [4,5]. The three kinases found in humans are Aurora kinase A (AURKA, also known as serine/threonine-protein kinase 6 or 15, breast tumor activated kinase, and Aurora-related kinase 1), Aurora kinase B (AURKB, also known as serine/threonine-protein kinase 5 and 12, protein

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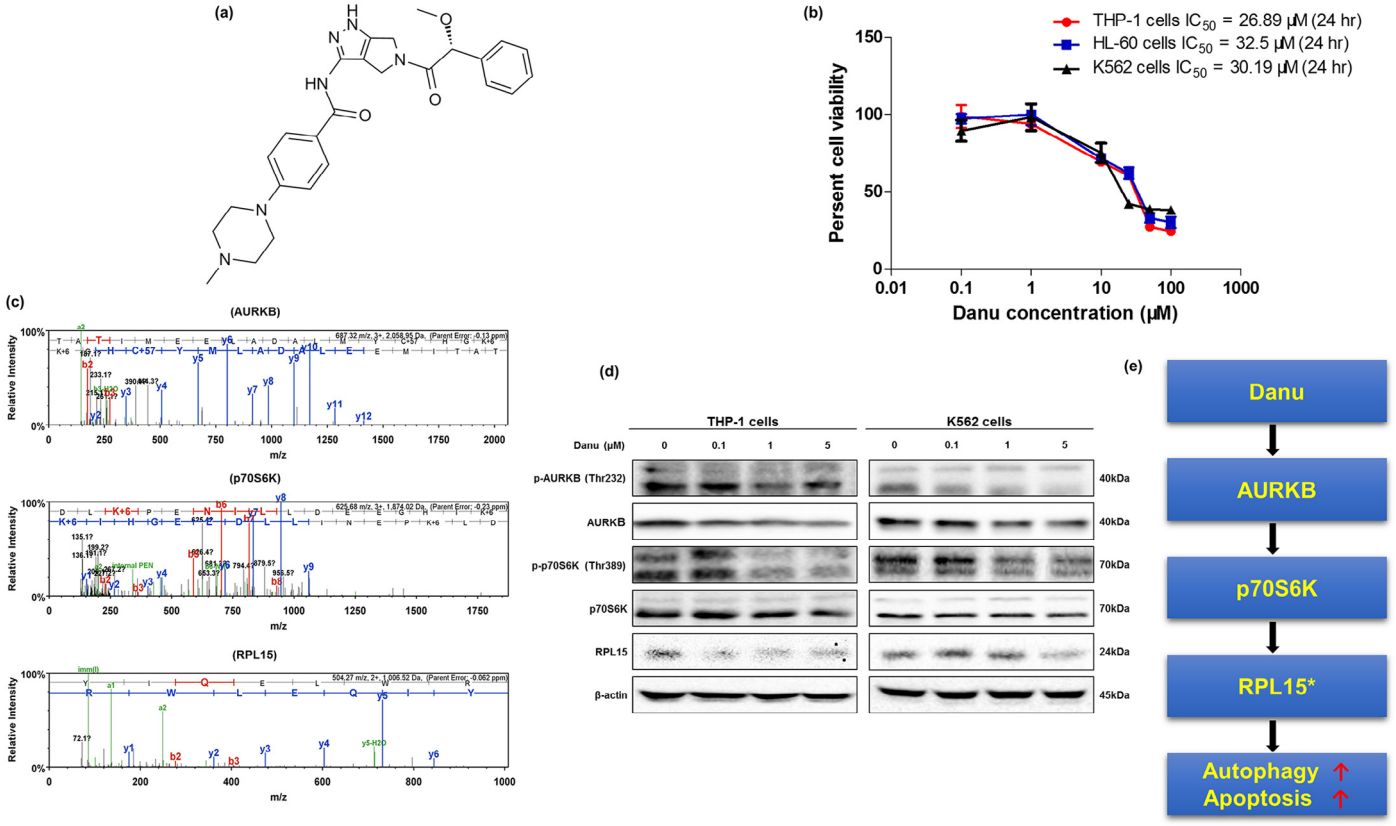

Fig. 1. Cytotoxic effect of Danu on human leukemia cells and the regulating effect on AURKB/p70S6K/RPL15 signal. (a) Chemical structure of Danu. (b) Effect of Danu on the viability of THP-1, HL-60, and K562 cells examined by the MTT assay. (c) Mass spectrum of AURKB, p70S6K, and RPL15 quantified and identified by the SILAC-based proteomics. (d) Representative blots of p-AURKA/B/C, AURKB, p-p70S6K, RPL15, and β-actin in THP-1, HL-60, and K562 cells when treated with Danu at 0.1, 1, and 5 μM for 24 h. (e) Proposed signaling pathways underlying the cell killing effect of Danu in THP-1, HL-60, and K562 cells.

phosphatase 1, and Aurora-related kinase 2), and Aurora kinase C (AURKC, also known as serine/threonine-protein kinase 13 and Aurora-related kinase 3) [4–6]. AURKA and AURKB act as essential regulators of mitosis and centrosome function by polymerizing microfilaments and controlling chromatid segregation, and AURKC may serve as a chromosomal passenger protein that can complement AURKB function in mitotic cells and support mitotic progression when AURKB is absent [7,8]. AURKA phosphorylates many target proteins and AURKB contributes to a number of processes that impart fidelity. Both AURKA and AURKB expression level is greatly increased in many human cancers including leukemia and lymphoma, which is linked to tumorigenesis, cancer metastasis, and poor prognosis [4,8,9]. Deregulation of Aurora kinase activity can result in mitotic abnormality and genetic instability, leading to defects in centrosome function, spindle assembly, chromosome condensation, microtubule-kinetochore attachment, and cytokinesis [10]. Therefore, Aurora kinases have emerged as attractive therapeutic targets for cancer treatment.

Currently, more than 35 small molecule inhibitors of Aurora kinases with differential Aurora kinase selectivity are in clinical Phase I and II trials including danusertib (formerly PHA-739358, Danu), (Fig. 1a), alisertib, barasertib, tozasertib, etc. Danu is developed by Nerviano Medical Sciences and is a pan-inhibitor of the Aurora kinases, with the IC_{50} values of 13, 79, and 61 nM for AURKA, AURKB, and AURKC, respectively [11–13]. Danu contains a pyrrolopyrazole scaffold which has been identified as an ATP-mimetic pharmacophore suited for kinase binding [13]. It induces apoptosis and autophagy in various cancer cell lines [14–16]. Danu has been investigated in several Phase I and II trials, showing a great therapeutic potential in the treatment of a variety of solid cancers [17–19]. In one Phase I study with 80 assessable cancer patients, stable disease was observed in 28 patients, and this lasted for six months in 7 patients. Phase II and Phase III single agent studies without G-CSF are underway in 7 types of solid tumors. Mice transplanted with human Bcr/Abl T315I ALL cells treated with a 3 × 7-day cycle of PHA-739358 as mono-treatment had significantly longer survival [20]. Danu also showed promising efficacy and safety in a Phase I dose escalation study in patients with accelerated or blastic phase chronic myeloid leukemia or Philadelphia chromosome-positive acute lymphoblastic leukemia [21].

Given the increasing incidence and poor response to current therapies for leukemia, it is requisite to discover and identify novel molecules and signaling pathways as potential therapeutic targets in leukemia treatment. On the other hand, with the exploration of novel targets of Danu, it can provide new clues for understanding exact mechanisms of action of Danu in cancer therapy. In this study, we further explored the molecular targets of Danu in leukemia cells using quantitatively SILAC-based proteomic analysis and the data were verified with a focus on apoptosis and autophagy-associated pathways.

Materials and methods

Chemicals and reagents

Danu was obtained from Selleckchem Inc. (Huston, TX, USA). $^{13}C_6$-L-lysine, L-lysine, $^{13}C_6{}^{15}N_4$-L-arginie, L-arginine, Dulbecco's modified Eagle's medium (DMEM)/F12 for SILAC, siRNA for (ribosomal protein L15 (RPL15), dimethy sulfoxide (DMSO), propidium iodide (PI), ribonuclease (RNase A), 2-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), bovine serum albumin, and Dulbecco's phosphate buffered saline (PBS) were purchased from Sigma-Aldrich (St Louis, MO, USA). SB202190 and LY294002 were obtained from InvivoGen Inc. (San Diego, CA, USA).