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Antibiotic bedaquiline effectively targets growth, survival and tumor angiogenesis of lung cancer through suppressing energy metabolism

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ABSTRACT

Tumor angiogenesis plays essential roles during lung cancer progression and metastasis. Therapeutic agent that targets both tumor cell and vascular endothelial cell may achieve additional anti-tumor efficacy. We demonstrate that bedaquiline, a FDA-approved antibiotic drug, effectively targets lung cancer cells and angiogenesis. Bedaquiline dose-dependently inhibits proliferation and induces apoptosis of a panel of lung cancer cell lines regardless of subtypes and molecular heterogeneity. Bedaquiline also inhibits capillary network formation of human lung tumor associated-endothelial cell (HLT-EC) on Matrigel and its multiple functions, such as spreading, proliferation and apoptosis, even in the presence of vascular endothelial growth factor (VEGF). We further demonstrate that bedaquiline acts on lung cancer cells and HLT-EC via inhibiting mitochondrial respiration and glycolysis, leading to ATP reduction and oxidative stress. Consistently, oxidative damage on DNA, protein and lipid were detected in cells exposed to bedaquiline. Importantly, the results obtained in *in vitro* cell culture are reproducible in *in vivo* xenograft lung cancer mouse model, confirming that bedaquiline suppresses lug tumor growth and angiogenesis, and increases oxidative stress. Our findings demonstrating that energy depletion is effectively against lung tumor cells and angiogenesis. Our work also provide pre-clinical evidence to repurpose antibiotic bedaquiline for lung cancer treatment.

1. Introduction

Lung cancer, mainly classified as non-small cell lung cancer and small cell lung cancer, remains the commonest form of cancer deaths in the world [1]. Surgery and cytotoxic chemotherapy are conventional therapies for lung cancer with poor cure rate [2,3]. Targeted therapies using epidermal growth factor receptor (EGFR) inhibitors, such as gefitinib and erlotinib, have revolutionized treatment for lung cancer. However, resistance to EGFR inhibitors still develops due to secondary EGFR mutation [4]. It is known that angiogenesis is important for lung cancer progression and metastasis. High levels of tumor angiogenesis has been demonstrated to be associated with poor prognosis in lung cancer patients [5,6]. Therefore, therapeutic agent that targets both tumor cell and vascular endothelial cell may represent a potential therapeutic strategy for lung cancer.

Bedaquiline is a FDA-approved antibiotic for the treatment of multi-drug resistant pulmonary tuberculosis [7]. The mechanism of its action on bacteria is ATP-synthase inhibition and subsequent mitochondrial dysfunction [8,9]. Similar to its action in bacteria, bedaquiline has been reported to inhibit mitochondrial respiration in cancer cells, leading to cancer cell growth arrest [10]. Besides glycolysis, many studies have shown that tumor cells, particularly tumor stem cells, are highly dependent on oxidative phosphorylation for expansion and survival [11–13]. In addition, mitochondrial inhibitors also effectively targets functions of endothelial cells, leading to inhibition of tumor angiogenesis in glioblastoma [14]. We therefore speculate that bedaquiline might target tumor cells and vascular endothelial cells.

In this study, we investigated the effect of bedaquiline on lung cancer cell growth, survival and angiogenesis. We show that bedaquiline inhibits lung cancer and angiogenesis *in vitro* and *in vivo*. We further show that the inhibitory effects of lung cancer

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are attributed to its inhibition of energy metabolism and induction of oxidative damage.

2. Materials and methods

2.1. Cell lines, primary endothelia cells, reagents and drugs

Multiple human lung cancer cell lines and primary human lung tumor-associated endothelial cells (HLT-EC) were purchased from American Type Culture Collection and Cell Biologics Inc. respectively. Cell lines were cultured using Dulbecco's Modified Eagle Medium (DMEM) (life technologies, US) containing 10% fetal bovine serum (FBS) (Hyclone, UK), 1 mM Sodium Pyruvate and 1% Penicillin-Streptomycin (Life technologies, US). HLT-EC were grown in Complete Human Endothelial Cell Medium. Recombinant human $VEGF_{165}$ (R&D Systems Inc. US) and bedaquiline (AdooQ Bioscience, US) were reconstituted in water and DMSO, respectively.

2.2. In vitro angiogenesis assays

In vitro angiogenesis assays, including capillary network formation, cell spreading and transwell migration, were performed using the same protocols as previously described in our past studies [15]. HLT-EC used for the experiments were from passages 2–4 and starved in Endothelial cell medium (ECM, served as the basal medium, Cell System, US) for 3 h before being treated with bedaquiline in the absence or presence of 15 ng/ml VEGF.

2.3. Measurement of proliferation and apoptosis

Cells were treated with different concentrations of bedaquiline for 72 h. Proliferation activity was measured by the CellTiter 96R Aqueous One Solution Cell Proliferation assay kit (Promega, US). Apoptosis was measured by staining of Annexin V-FITC/7-AAD (Abnova Inc, US) followed by flow cytometry analysis on a Beckman Coulter FC500. The percentage of Annexin V-positive cells was determined by CXP analysis.

2.4. Mito stress and glycolysis stress test assays

Cells were treated with different concentrations of bedaquiline for 24 h. Mito stress assay measures the oxygen consumption rate (OCR), an indicator of mitochondrial respiration, under basal and maximal conditions in cells. Glycolysis stress test measures the extracellular acidification rate (ECAR), an indicator of glycolytic function. After drug treatment, media were replaced by XF assay medium (Seahorse Bioscience, US) and incubated at 37 °C in a non-CO2 incubator for 1 h. All injection reagents were adjusted to pH 7.4 on the day of the assay. The subsequent mito stress or glycolysis stress assay was conducted as per the Seahorse XF24 analyzer standard protocol. The Seahorse XF-24 software calculated OCR and ECAR automatically.

2.5. Measurement of cellular ATP and mitochondrial superoxide levels

Cells were treated with different concentrations of bedaquiline for 24 h. ATP levels were measured by ATPlite Luminiescent Assay kit (Perkin Elmer, US) according to the manufacturer's protocol. Mitochondrial superoxide was measured by incubating cells with 5 μM MitoSox Red at 37 °C for 20 min. The absorbance at ex/em of 510/580 nm were measured using Spectramax M5 microplate reader (Molecular Devices).

2.6. Measurement of cellular oxidative damage

Cells were treated with different concentrations of bedaquiline for 24 h. DNA was extracted using the DNEasy Mini Kit (Qiagen, US). Oxidative DNA damage was determined by measuring 8-hydroxy-2′-deoxyguanosine (8-OHdG) levels using the OxiSelect Oxidative DNA Damage ELISA Kit (Cell Biolabs). Oxidative protein damage was determined by measuring protein carbonylation using the Protein Carbonyl ELISA Kit (Enzo LifeSciences). Oxidative lipid damage was determined by measuring lipid peroxidation using the Lipid Peroxidation MDA Assay Kit (Abcam, US). All assays were performed strictly according to manufacturer's protocols.

2.7. Lung xenograft mouse tumor in SCID mouse and immunohistochemistry

All procedures were conducted according to the guidelines approved by the Institutional Animal Care and Use Committee. A549 cells (10^6) suspended in 100 μl PBS were injected into the flank of 6 weeks old SCID mice. After development of palpable tumors, the mice with similar tumor size were divided into two groups and treated with intraperitoneal bedaquiline at 10 mg/kg daily and the same amount of 20%/80% DMSO/Saline as control. Tumor length and width were measured every three days and all mice were euthanized after the control tumor reached a size of more than 1 cm or showed necrosis. Tumor frozen section slides were fixed with 4% paraformaldehyde (Sigma, US). The slides were incubated with primary antibodies against CD31 and 4-HNE (Cell Signaling, US), and then secondary antibody conjugated with horseradish peroxidase-DAB (3, 3′- diaminobenzidine). The nuclei were counterstained with hematoxylin (Sigma, US).

2.8. Statistical analyses

All data are expressed as mean and standard deviation (SD) to indicate data variability. Statistical analyses were performed by unpaired Student's *t*-test. A p-value < 0.05 was considered statistically significant.

3. Results

3.1. Bedaquiline inhibits proliferation and induces apoptosis of a panel of lung cancer cell lines in vitro

Lung cancer cell lines, derived from patients with lung adenocarcinoma, have been widely used as an *in vitro* model for lung cancer, particularly in determining in drug sensitivity and for the understanding of mechanisms of drug action [16]. We firstly investigate the effects of bedaquiline on the proliferation and apoptosis in a panel of lung cancer cell lines, including PC-9, A549, NCI-H69, DMS-53, H157 and EBC-1. These cell lines presents molecular heterogeneity and main subtypes of lung cancer, such as small cell lung cancer and non small cell lung cancer. We found that bedaquiline significantly inhibited proliferation of all tested cell lines in a concentration-dependent manner (Fig. 1A). The IC50 range of bedaquiline is 9–12 μM. In addition, bedaquiline significantly induced apoptosis of these lung cancer cell lines (Fig. 1B). These results suggest that bedaquiline is active against lung cancer cells regardless of subtypes and molecular heterogeneity.

3.2. Bedaquiline significantly inhibits lung tumor angiogenesis and various functions of HLT-EC in vitro

Given the important roles of angiogenesis in the development, progression and metastasis of lung cancer [17,18], we next

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