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The anti-malarial atovaquone selectively increases chemosensitivity in retinoblastoma via mitochondrial dysfunction-dependent oxidative damage and Akt/AMPK/mTOR inhibition

Feng Ke [a], Jinqiang Yu [a], Wei Chen [b], Xiaomin Si [c], Xinhui Li [d], Fang Yang [a], Yingying Liao [e, *], Zhigang Zuo [f, **]

[a] Department of Ophthalmology, Renmin Hospital, Hubei University of Medicine, Shiyan, China
[b] Department of Critical Care Medicine, Taihe Hospital, Hubei University of Medicine, Hubei, China
[c] Department of Cardiopulmonary Rehabilitation, Renmin Hospital, Hubei University of Medicine, Shiyan, China
[d] Department of Oncology, Shiyan Taihe Hospital, Shiyan, China
[e] Department of Gastroenterology, Renmin Hospital, Hubei University of Medicine, Shiyan, China
[f] Department of Oncology, Renmin Hospital, Hubei University of Medicine, Shiyan, China

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ABSTRACT

Mitochondria has been identified as a promising target in several cancers. However, little is known on the effects of targeting mitochondria in retinoblastoma. In this work, we show that anti-malarial atovaquone, at clinically achievable concentration, demonstrates inhibitory effects to retinoblastoma cells, to a more extent than in normal retinal cells. Atovaquone also significantly increases chemosensitivity in retinoblastoma. Importantly, we show that retinoblastoma cells have higher level of mitochondrial respiration, membrane potential, mass and ATP compared to normal retinal cells. Although atovaquone significantly inhibits mitochondrial respiration and decrease ATP level in both malignant and normal retinal cells in a similar manner, atovaquone induces much more oxidative stress and damage in retinoblastoma than normal retinal cells. These suggest that normal retinal cells are more tolerable to mitochondrial dysfunctions than retinoblastoma cells. We further demonstrate that atovaquone targets Akt/AMPK/mTOR signaling via inducing mitochondrial dysfunction. Our pre-clinical work demonstrates the translational potential of atovaquone as an addition to the treatment armamentarium for retinoblastoma. Our work also demonstrates the differences of mitochondrial biogenesis and function in malignant versus normal retinal cells which are important for the targeted therapy in retinoblastoma.

1. Introduction

Retinoblastoma is an ocular cancer of retinal origin and typically occurs children younger than 5 years. Although the standard treatment including enucleation, chemotherapy and radiation significantly improves clinical outcome, the management of retinoblastoma is still challenge in developing countries [1]. Mutation of the tumor suppressor gene retinoblastoma 1 (RB1) plays a central role in retinoblastoma transformation and progression [2,3]. Substantial evidence has shown extensive heterogeneity in retinoblastoma at molecular, cellular and tumor level [4], suggesting that targeting common cancer drivers may represent a potential therapeutic strategy for retinoblastoma.

The mitochondria is an important energy and biosynthetic factories supporting cancer cell growth and survival [5]. Apart from energy metabolism, other functions of mitochondria include calcium homeostasis, redox regulation and apoptosis [6]. Importantly, studies have recently shown that cancer cells have increased mitochondrial biogenesis and rely more on mitochondrial respiration compared to normal cells [7–9]. Inhibition of mitochondrial functions display anti-cancer activities while having less toxicity to normal counterparts [10]. Targeting mitochondria using clinically available drugs as a cancer therapeutic strategy has attracted much

* Corresponding author. Department of Gastroenterology, Renmin Hospital, Hubei University of Medicine, Maojian District, Chaoyangzhong Road 39, 442000, China.
** Corresponding author. Department of Oncology, Renmin Hospital, Hubei University of Medicine, Maojian District, Chaoyangzhong Road 39, 442000, China.
E-mail addresses: 105806770@qq.com (Y. Liao), doctor_zzg@163.com (Z. Zuo).

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attention in the recent years and shown translational potential in pre-clinical and clinical settings [8,11].

Atovaquone is an anti-malarial drug with its target on cytochrome bc1 complex and therefore affecting mitochondrial function [12,13]. In this study, we investigated the effect and target of atovaquone in retinoblastoma (malignant) and normal retinal cells. We further investigated the basal mitochondrial biogenesis of malignant and normal retinal cells to understand their differential response to atovaquone. Our findings demonstrate that inhibition of mitochondrial respiration as a potential targeted strategy for retinoblastoma.

2. Materials and methods

2.1. Primary cells, cell culture and generation of ρ0 cell line

Human retinoblastoma cell lines RB116 (Kerafast Inc. US), Y79 and WERI-Rb-1 (ATCC, US) and immortalized normal retinoblastoma pigmented epithelial cell line (RPE-1, ATCC, US) were grown in RPMI 1640 media supplemented with 10% fetal bovine serum, 2 mM glutamine, 1 mM sodium pyruvate, and 4.5 g/L glucose. Primary normal retinoblastoma pigmented epithelial cell (HNRPE, Abm, US) was grown in extracellular matrix (Abm, US)-coated flask using the media (Abm, Cat. No. TM4057) according to manufacture's instruction. Mitochondria DNA-deficient ρ0 cell line was established according to the method previously described [14]. The lack of mitochondrial DNA in ρ0 cells was confirmed using reverse transcription–PCR (data not shown).

2.2. Migration assays

Migration assay was performed using the Boyden chamber which consists a Falcon cell culture insert and 24-well plate chamber. Cells (10,000/well) and atovaquone were added into the cell culture insert. 20% FBS was placed into the lower chamber. The assembled cell culture insert chamber was incubated for 6–8 h. Cells on the upper surface of the insert were then removed with a cotton swab. Migratory cells on the lower surface of inserts were fixed with 4% formaldehyde (Sigma, US) for 10 min, then stained with 0.4% Giemsa and counted under using light microscope (Zeiss, Germany).

2.3. Proliferation assay

Cells (10, 000/well) were treated with atovaquone, vincristine or combination for 72 h in 96-well-plate. Proliferation was measured using the CellTiter 96 AQueous One Solution Cell Proliferation assay kit (Promega, US).

2.4. Mito stress and glycolytic stress test assays

Cells (10,000/well) were seeded, cultured and treated with atovaquone in XF96 cell culture plates. After treatment, media were replaced by XF assay medium (Seahorse Bioscience, US) and incubated at 37 °C in a CO_2-free environment for 1 h. Oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) were measured at 37 °C using an XF96 extracellular analyzer

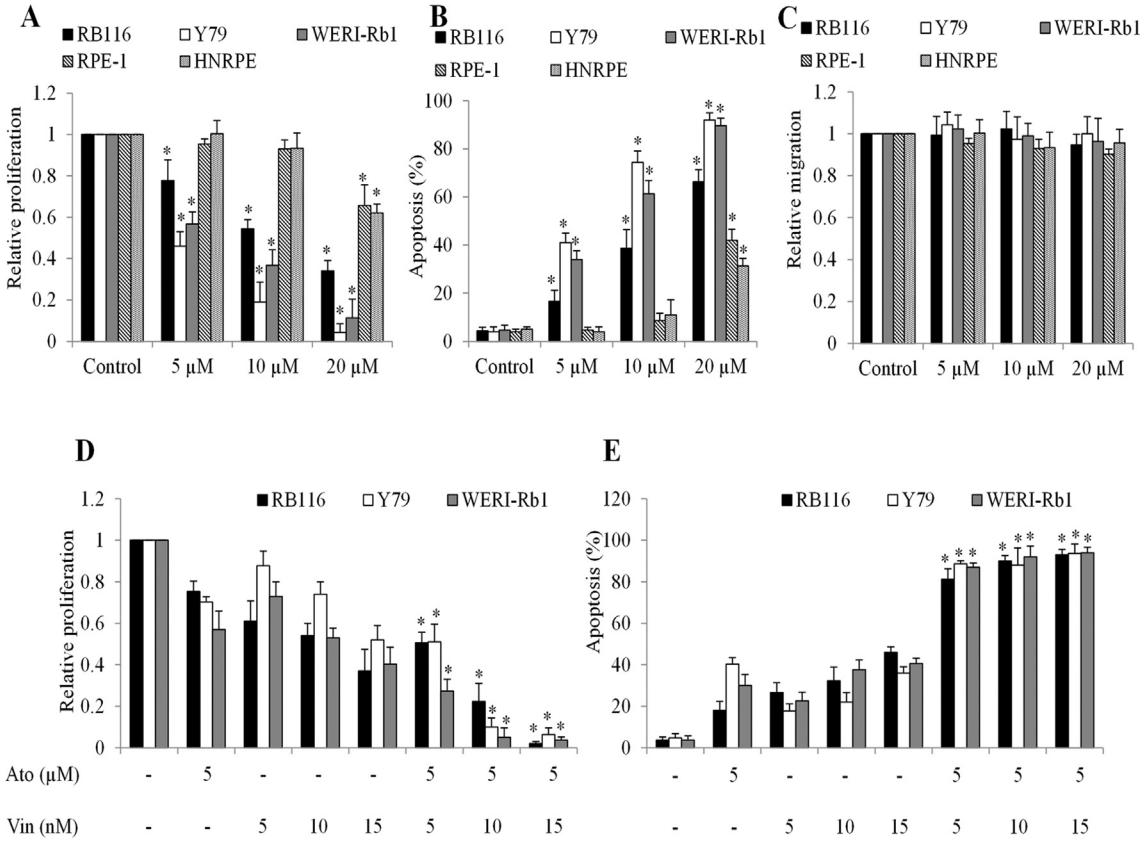

Fig. 1. Atovaquone selectively targets retinoblastoma cells and enhances the inhibitory effects of chemotherapeutic agent. Atovaquone is more effective in inhibiting proliferation (A) and inducing apoptosis (B) in retinoblastoma cells: RB116, Y79 and WERI-Rb1 cells than normal retinal cells: RPE-1 and HNRPE. Atovaquone significantly enhances the anti-proliferative (C) and pro-apoptotic (D) effects of vincristine in retinoblastoma cells. Atovaquone at 5 μM was used in combination studies. *$p < 0.05$, compared to control or vincristine alone.

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