Biomedicine & Pharmacotherapy 85 (2017) 479–485

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Original article

Erlotinib has tumor inhibitory effect in human retinoblastoma cells

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ARTICLE INFO

Keywords:
Retinoblastoma
Erlotinib
Cancer proliferation
Cancer migration
Cancer graft

ABSTRACT

Aim: In this study, we explored the effect of erlotinib on the development of retinoblastoma (RB) cells both *in vitro* and *in vivo*.
Method: RB cell lines, Y79 and WERI cells were treated with various concentrations of erlotinib *in vitro* to assess their cytotoxic profiles. *In vitro* proliferation, cell-cycle transition and migration were compared between RB cells treated with erlotinib and cells without erlotinib treatment. In *in vivo* tumorigenicity assay, mice were injected with Y79 cells and orally fed with erlotinib for 28 days. The effect of erlotinib on *in vivo* tumor grafts was then assessed. Western blot analysis on EGFR, ERK, AKT proteins and their phosphorylated proteins was also performed to assess molecular signaling pathways of associated with erlotinib in RB cells.
Results: *In vitro* erlotinib treatment induced cytotoxicity in Y79 and WERI cells in dose-dependent manner. While Y79 and WERI cells were treated with erlotinib close to EC_{50} concentrations for 3 days, RB proliferation, cell-cycle transition and migration were all significantly inhibited. In *in vivo* tumorigenicity assay, oral induction of erlotinib also dramatically reduced the growth of Y79 tumor grafts. Western blot demonstrated that, in *in vitro* RB cells, erlotinib did not alter the protein expression levels of EGFR, ERK or AKT, but significantly reduced the expressions of phosphorylated EGFR, ERK and AKT proteins.
Conclusion: Erlotinib was shown to have tumor suppressive effect on RB growth *in vitro* and *in vivo*, possibly through the inhibition on EGFR, ERG/AKT signaling pathways.

1. Introduction

Retinoblastoma (RB) is one of the most malign ophthalmologic tumors in young or infant patients [1–3]. Most of the RB patients, once diagnosed, were already at late stages, thus suffered with poor clinical outcomes and survival rates of less than 50% [4]. During the past decade, the strategy to manage RB progression has been shifted from radiology to chemotherapy [5,6]. However, for most of the chemotherapy treatment plans, such as intravitreal chemotherapy, intraarterial chemotherapy, intravenous chemotherapy or periocular chemotherapy, patients still had high rates of adverse events and little improvement on overall survival [5,7]. Therefore, there is a great need to elucidate the underling molecular mechanisms of RB development, and develop novel chemotherapeutic targets to treat patients with retinoblastoma.

Studies have demonstrated that epidermal growth factor receptors (EGFRs) played critical roles in carcinoma development and apoptosis in various human cancers [8,9]. Overexpression of EGFR or phosphorylated EGFR (p-EGFR) has significant oncogenic effect, whereas inhibition of EGFR or EGFR phosphorylation induces apoptosis among carcinomas, thus making EGFR a potential therapeutic target for cancer treatment [8–10]. In human disease of RB, a very recent study demonstrated, through epigenetic regulation of microRNA, EGFR signaling is associated with cancer cell proliferation [11]. However, little is known whether EGFR signaling pathway is directly involved in the regulation of RB.

During the past decade, several tyrosine kinase inhibitors (TKIs), such as erlotinib and lapatinib, have been shown to effectively target EGFR to inhibit oncogenic signaling transduction cascade, thus been approved by USFDA to treat patients with non-small cell lung cancer

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http://dx.doi.org/10.1016/j.biopha.2016.11.054
0753-3322/

and breast cancer [12,13]. In addition, while combined with trastuzumab, erlotinib was shown to have tumor suppression on breast cancer development both *in vitro* and *in vivo* [14,15].

In this study, we investigated the effects of erlotinib on the development of human RB cells. In the culture of Y79 and WERI cells, we examined their cytotoxic profiles in response to *in vitro* erlotinib application. We also examined the possible tumor suppression of erlotinib on *in vitro* RB proliferation, cell-cycle transition, migration, and *in vivo* RB grafts in mice tumor transplantation model. Furthermore, we used western blot to assess the effect of erlotinib on EGFR, ERK/AKT signaling pathways in RB cells.

2. Materials and methods

2.1. Ethic statement

All work was approved by the Ethic Committees at the First Affiliated Hospital of Nanchang University, Jiangxi Province clinical ophthalmology Institute, The Third Hospital of Nanchang, and Fujian Provincial Key Laboratory of Ophthalmology and Visual Science.

2.2. Cell culture

Two human retinoblastoma cell lines, Y79 and WERI cells were purchased from Cell Bank of Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). All cells were maintained in 6-well plates with RPMI-1640 cell culture medium (Thermo Fisher Scientific, USA) supplemented with 10% heat-inactivated fetal bovine serum (HI-FBS, Thermo Fisher Scientific, USA) at 37 °C with 5% CO_2. Cells were passaged every 3–5 days when they reached ~80% confluency.

2.3. Cytotoxicity assay

During *in vitro* culture, Y79 and WERI cells were treated with Erlotinib (Tarceva®, Roche, USA) at various concentrations (0.001–100 μM) for 72 h to assess their cytotoxic response to Erlotinib. Cell viability was evaluated using a 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay (Thermo Fisher Scientific, USA) according to manufacturer's recommendations, and normalized to the absorbance (at 570 nm) measured for cells treated with 0 μM Erlotinib.

2.4. Proliferation assay

Y79 and WERI cells were removed from 6-well plate, re-suspended and reseeded in 96-well plates at low density (2500 cells/well). Y79 cells were incubated with 1 μM Erlotinib, and WERI cells were incubated with 2 μM Erlotinib. Control cells were not treated with Erlotinib (Blank, vehicle only). They were maintained for 5 consecutive days. Cell proliferation was evaluated

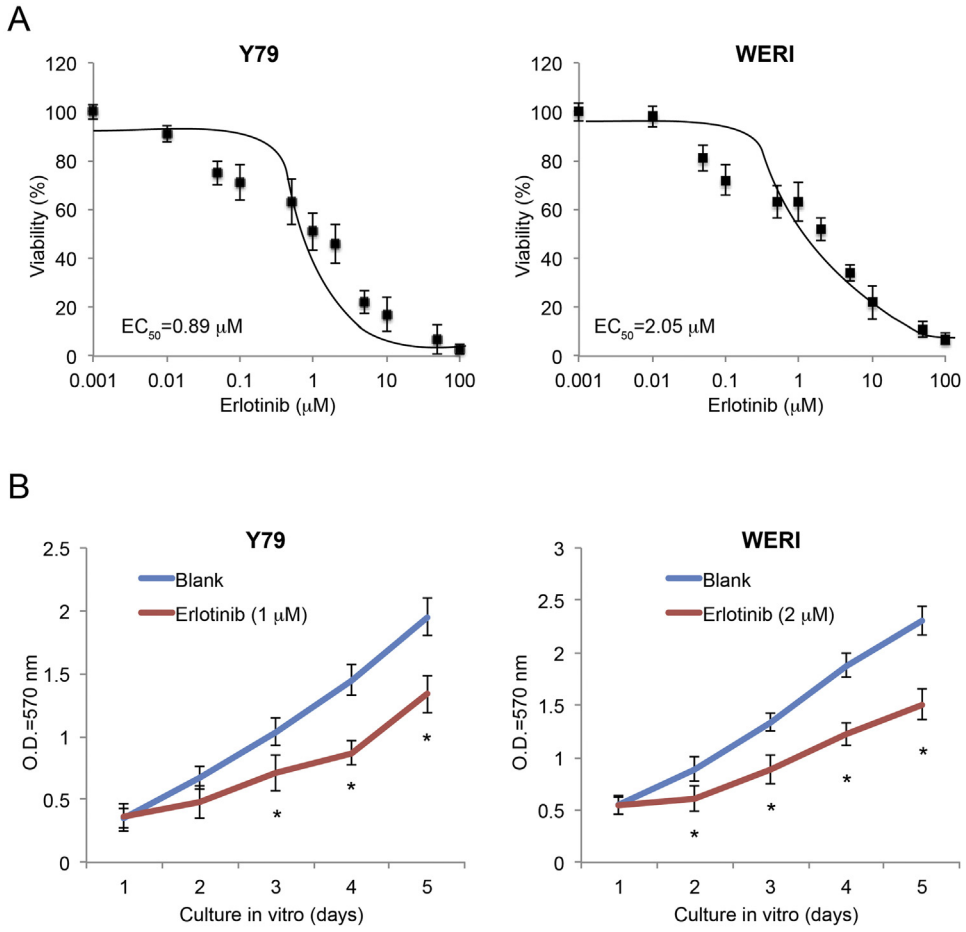

Fig. 1. Erlotinib has tumor suppressive effect on retinoblastoma progression *in vitro*. **(A)** RB cell lines Y79 and WERI cells were incubated with erlotinib (0.001, 0.01, 0.05, 0.1, 0.5, 1, 2, 5, 10, 50, 100 μM) for 72 h *in vitro*. Cell viability was assessed by a MTT assay and fitted by a Hill equation to determine EC_{50} concentrations (n = 8). **(B)** Y79 cells were incubated with 1 μM erlotinib (n = 6), WERI cells with 2 μM erlotinib (n = 6), or no erlotinib (Blank, n = 12) for 5 days *in vitro*. RB proliferation was measured by a MTT assay and compared between Blank and erlotinib treatments (* $P < 0.05$).