Biochemical and Biophysical Research Communications xxx (2015) 1–6

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

The bisphosphonate zoledronic acid effectively targets lung cancer cells by inhibition of protein prenylation

Fan Xie [a], Pengcheng Li [b], Jianhua Gong [a], Jiahong Zhang [a], Jingping Ma [a, *]

[a] *Department of Respiratory Medicine, Jingzhou Hospital, Tongji Medical College, Huazhong University of Science and Technology (HUST), Jingzhou, PR China*
[b] *Department of Oncology, Wuhan Union Hospital Affiliated to Huazhong University of Science and Technology, Wuhan, PR China*

ARTICLE INFO

Article history:
Received 11 October 2015
Accepted 18 October 2015
Available online xxx

Keywords:
Lung cancer
Bisphosphonate
Zoledronic acid
Prenylation
Ras signalling

ABSTRACT

Aberrant activation of oncoproteins such as members of the Ras family is common in human lung cancers. The proper function of Ras largely depends on a post-translational modification termed prenylation. Bisphosphonates have been shown to inhibit prenylation in cancer cells. In this study, we show that zoledronic acid, a third generation bisphosphonate, is effective in targeting lung cancer cells. This is achieved by the induction of apoptosis and inhibition of proliferation, through suppressing the activation of downstream Ras and EGFR signalling by zoledronic acid. The combination of zoledronic acid and paclitaxel or cisplatin (commonly used chemotherapeutic drugs for lung cancer) augmented the activity of either drug alone in *in vitro* lung cancer cellular system and *in vivo* lung xenograft mouse model. Importantly, zoledronic acid inhibits protein prenylation as shown by the increased levels of unprenylated Ras and Rap1A. In addition, the effects of zoledronic acid were reversed in the presence of geranylgeraniol and farnesol, further confirming that mechanism of zoledroinc acid's action in lung cancer cells is through prenylation inhibition. Since zoledronic acid is already available for clinic use, these results suggest that it may be an effective addition to the armamentarium of drugs for the treatment of lung cancer.

© 2015 Elsevier Inc. All rights reserved.

1. Introduction

Lung cancer represents the leading cause of cancer death worldwide, and non-small-cell lung cancer (NSCLC) and small-cell lung cancer (SCLC) account for the majority of lung cancer cases [1]. Although activation of epidermal growth factor receptor (EGFR) play an essential role in lung carcinogenesis and targeted therapies blocking EGFR have shown efficacy in clinical practice, patients still develop resistant [2]. Besides EGFR mutations, the essential mechanisms of secondary resistance are the overexpression and activation of Ras onco-proteins in the development of lung cancer [3,4].

The proper function of Ras requires processing via the prenylation, a post-translational modification that anchors the proteins to the plasma cell membrane, for binding to effector molecules in the various downstream signalling pathways [5]. Prenylation is catalyzed by farnesyltransferase (FTase) and geranylgeranyltransferase (GGTase). Recent research have found that N-Ras and K-Ras can be geranylgeranylated even in the presence of FTase inhibition [6]. For this reason, the blockade of Ras activation requires the inhibition of both FTase and GGTase.

Bisphosphonates (BP) are structurally analogues of the endogenous pyrophosphate [7]. BP prevent the prenylation of GTP-binding proteins (eg. Ras), via blocking key enzyme of the mevalonate pathway farnesyl diphosphate synthase, thereby inhibition of both farnesylation and geranylgeranylation [8]. Zoledronic acid (ZA), a third generation BP, has been shown to induce apoptosis, inhibit cell cycle progression of several types of cancer cells *in vitro* and *in vivo* [9–11]. Recently, ZA was proven to enhance the effect of EGFR inhibitors and overcome resistance in EGFR family-driven cancers [12].

In this study, we investigated the effect of ZA and whether its combination with the chemotherapeutic drugs paclitaxel and cisplatin offers any advantage for elimination of lung cancer cells. We also investigated the effect of ZA on prenylation, downstream signalling pathways of Ras and EGFR in lung cancer cells. Our

* Corresponding author. Department of Respiratory Medicine, Jingzhou Hospital, Tongji Medical College, Huazhong University of Science and Technology (HUST), Renmin Road 1, 434020, PR China
E-mail address: mjpjzhospital@hotmail.com (J. Ma).

http://dx.doi.org/10.1016/j.bbrc.2015.10.089
0006-291X/© 2015 Elsevier Inc. All rights reserved.

ARTICLE IN PRESS

results reveal that ZA alone is effective in targeting lung cancer cells via prenylation inhibition, suppression of phosphorylation of Ras and EGFR signalling. The combination of ZA with chemotherapeutic drugs is synergistic *in vitro* and *in vivo*. Our study also emphasize the potential therapeutic value of targeting protein prenylation in human lung cancer treatment.

2. Materials and methods

2.1. Cells and drugs

Human lung cancer cell lines A549, PC-9, NCI-H69 and DMS-53 were purchased from American Type Culture Collection (ATCC) and grown in Dulbecco's Modified Eagle Medium (DMEM) (life technologies, US) containing 10% fetal bovine serum (FBS) (Hyclone, UK). Zoledronic acid (ZA; Sigma US) was dissolved in water. Geranylgerniol (GGOH), farnesol (FOH), paclitaxel and cisplatin were purchased from Sigma and dissolved in DMSO.

2.2. MTS assay

Cell proliferation activity was evaluated by the CellTiter 96 AQueous One Solution Cell Proliferation assay kit (Promega, US). Briefly, after 3 days drug treatment, 20 μl of CellTiter 96 AQueous One Solution were added to each well and the plates were incubated for a further 2–4 h at 37 °C. Absorbance was measured at 490 nm.

2.3. Measurement of apoptosis

Cells were treated with drugs for 3 days, were then stained with Annexin V-FITC and propidium iodide (PI). The stained cells were analysed on a Beckman Coulter FC500. The percentage of Annexin V-positive cells was determined by CXP software analysis.

2.4. Denaturing sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) and western blot (WB) analyses

Cells were treated with drugs for 24 or 48 h and then were lysed by RIPA buffer (Life Technologies Inc, US) for total protein. Proteins (50 μg) from whole cell extracts were resolved using denaturing SDS–PAGE and analysed by WB. Antibodies used in WB analyses include anti-PCNA, anti-RAPR, anti-p-AKT(ser473), anti-t-AKT, anti-p-S6, anti-t-S6, anti-p-Erk(Thr202/Tyr204), anti-t-Erk, anti-p-STAT3(Tyr705), anti-t-STAT3 and anti-β-actin (Cell Signalling Technologies, US), anti-Ras (Becton Dickinson) and anti-Rap1A (Santa Cruz Biotechnology, US). The bands were detected using the chemiluminescence kits (Amersham Biosciences, UK). Immunoblots shown are the representative of three independent experiments.

2.5. Lung cancer xenograft in SCID mouse

SCID mice at 8 weeks old were purchased from Animal Resources Centre Australia. All of the animal experiments were approved by the Institutional Animal Care and Use Committee of HuaZhong University of Science and Technology. One million A594 cells were suspended in 1XPBS and subcutaneously injected into the right flank of each mouse. Ten mice were randomized into each group. When tumour volume reached approximately 200 mm^3, the mice were treated with vehicle control, intraperitoneal zoledronic acid, paclitaxel or cisplatin at 10 mg/kg once daily, combination of zoledronic acid with paclitaxel or cisplatin. Tumour length and width were measured every three days and the volumes were calculated using the formula: length $\times$ width^2 $\times$ 0.5236.

2.6. Statistical analyses

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

3. Results

3.1. ZA significantly inhibits proliferation and induces apoptosis of multiple lung cancer cell lines

We firstly investigated the effects of ZA on the proliferation and apoptosis in multiple lung cancer cell lines, including A549 and PC9 (derived from NSCLC patients) as well as NCI-H69 and DMS-53 (derived from the SCLC patients). We found that ZA dose-dependently inhibited proliferation of lung cancer cell lines as assessed by MTS assay (Fig. 1A). The effector concentration for half-maximal response (EC50) of ZA to lung cancer cell lines is similar (around 10 μM) (Fig. 1A), suggesting that both NSCLC and SCLC lines are sensitive to ZA. In addition, ZA significantly induced apoptosis in lung cancer cell lines as assessed by flow cytometry for Annexin V staining (Fig. 1B). Decreased level of proliferating marker protein PCNA and increased level of apoptotic protein PARP (cleaved form) were also observed in lung cancer cell lines treated with ZA (Fig. 1C), further demonstrating the anti-proliferative and pro-apoptotic effects of ZA in lung cancer cells.

3.2. ZA targets lung cancer cells through inhibition of prenylation

In order to confirm that ZA's anti-proliferative and pro-apoptotic effects on lung cancer cell lines were associated with its capacity to inhibit post-translational modification of small GTP-binding proteins, we analysed the prenylation status of Ras and Rap1A. Ras can be prenylated by both FTase and GGTase and inhibition of prenylation can be detected by immunoblotting because its unprenylated forms display reduced mobility in SDS-PAGE compared with their prenylated. In contrast, Rap1A is a protein prenylated exclusively by GGTase 1 [13], and its unprenylated form is recognized by a specific unprenylated anti-Rap1A antibody.

A549, PC-9, NCI-H69 and DMS-53 were treated with ZA for 48 h. In control-treated lung cancer cell lines, Ras was in the processed, prenylated forms. However, treatment with ZA inhibited the processing of Ras, resulting in unprenylated protein forms with reduced electrophoretic mobility (Fig. 2A). Consistently, the increased level of unprenylated Rap1A was also detected in lung cancer cell lines treated with ZA (Fig. 2A).

Geranylgeraniol (GGOH) is metabolized to geranylgeranyl pyrophosphate (GGPP) in the cells to restore geranylgeranylation of Ras [14], but has no effect on the inhibition of the farnesylation of Rap1A. In contrast, farnesol (FOH) is metabolized to farnesyl pyrophosphate (FPP) to restore farnesylation but not Ras geranylgeranylation. In our study, we found that the effects of ZA was partially reversed by the addition of GGOH or FOH alone (Fig. 2B and C). However, in the presence of both GGOH and FOH (Fig. 2B and C), the effects of ZA were further reversed. These data clearly demonstrate that ZA inhibits protein prenylation through the dual action of ZA on FTase and GGTase.

3.3. ZA suppresses downstream signalling of Ras and EGFR in lung cancer cells

Ras oncoproteins are activated in lung cancer and control the two crucial growth and survival signalling cascades: PI3K (the phosphoinositide 3-kinase)-Akt-mTOR and Ras-RAF-MEK (extracellular signal-related kinase) pathway. To explore the effects of ZA

Please cite this article in press as: F. Xie, et al., The bisphosphonate zoledronic acid effectively targets lung cancer cells by inhibition of protein prenylation, Biochemical and Biophysical Research Communications (2015), http://dx.doi.org/10.1016/j.bbrc.2015.10.089

Download English Version:

https://daneshyari.com/en/article/10750560

Download Persian Version:

https://daneshyari.com/article/10750560

Daneshyari.com