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Pharmacological synergism of 2,2-dichloroacetophenone and EGFR-TKi to overcome TKi-induced resistance in NSCLC cells

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Erlotinib (PubChem CID: 176870)
Gefitinib (PubChem CID: 123631)
Sodium dichloroacetate (PubChem CID: 517326)
2,2-dichloroacetophenone (PubChem CID: 72870)

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ABSTRACT

Combination treatment has been used as one of the therapeutic approaches for patients suffering from lung cancer, either to cope with the issue of acquired drug resistance due to prolong the use of a particular EGFR-TKi treatment, or to decrease the doses of each compound in order to reduce potential toxicity. 2,2-dichloroacetophenone (DAP) was reported as a PDK inhibitor recently, which is much more potent than dichloroacetate (DCA) in anti-cancer therapy. In this study, we applied DAP in combined with EGFR-TKis, erlotinib or gefitinib in NSCLC cell lines and NSCLC xenograft model. Synergistic anti-cancer effects in two NSCLC cell lines with EGFR mutation, NCI-H1975 and NCI-H1650, as well as in NCI-H1975 xenograft model were observed. In comparison with either DAP or EGFR-TKi applied alone, the combination treatment not only further suppressed the EGFR signaling *in vitro* and *in vivo*, but also significantly promoted cell apoptosis. Interestingly, this synergistic anti-cancer effect was also observed in NCI-H1975 gefitinib induced-resistant cell line. Taken together, our results suggested that the combined use of DAP and EGFR-TKi exhibited anti-cancer synergy which may offer an additional treatment option for patients with EGFR-TKi induced-resistance.

1. Introduction

Lung cancer is the most prevalent malignancy worldwide (Torre et al., 2015). Traditional therapeutic strategies, such as platinum-based chemotherapies, remain to be the first-line systematic therapies for patients with advanced or metastasis lung cancer (NCCN guideline, 2016), and offer clinical benefits such as controlling the disease progression and improving the quality-of-life (Ismael et al., 2008). Yet, targeted therapy is gaining ground for patient subgroups with certain phenotypes of cancer in the context of personalized therapy (Heng, 2016). Around 30–40% of non-small cell lung cancer (NSCLC) cases are of epidermal growth factor receptor (EGFR) mutation phenotype (Passaro et al., 2016). In particular, EGFR mutation leads to over-activation of its down-stream signaling transduction, which enables cancer cell survival, proliferation and differentiation (Zhang et al., 2010). To this end, EGFR specific tyrosine kinase inhibitors (TKis) were developed for these patients and made tremendous success in recent years (Lynch et al., 2004).

However, prolong exposure to EGFR-TKi could inevitably lead to acquired resistance in NSCLC patients (Pao et al., 2010), which could be due to a second-site hot spot mutation commonly occurred at EGFR T790 (Kobayashi et al., 2005). To overcome this issue, the third-generation EGFR-TKis represented by AZD9291 was developed (Cross et al., 2014). Moreover, activation of other bypass signaling pathways was found to contribute to acquired resistance, such as amplification of mesenchymal-epithelial transition (MET), one of the human hepatocyte growth factor receptor (Bean et al., 2007), and mutation of phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K) pathways (Kawano et al., 2006). Combination treatment has emerged as a promising strategy to deal with acquired resistance. Nonetheless, there is no MET or PI3K inhibitors available in the market except idelalisib, which targets PI3K delta isoform for severe lymphoma therapy (Anastasia et al., 2016; Franke et al., 1997), whereas the effectiveness of targeted therapy in combined with EGFR-TKi should be accessed on a case by case basis. Diverse signaling activations might be developed in different patients who showed resistance to EGFR-TKi (Sequist et al., 2011; Sano et al., 2015). Combinations of EGFR inhibitors with classic cytotoxic chemotherapy had been evaluated (Matikas et al., 2015). Disappointingly, meta-analysis showed that these combinations were far from satisfactory, as they did not bring any significant clinical benefits while causing severe toxic reactions for patients involved in those clinical trials (Yan et al., 2015). This highlights an urgent need for effective and safe combination strategies to overcome acquired resistance in EGFR-TKi therapy.

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In our previous study, dichloroacetate (DCA), the first and well-regarded pyruvate dehydrogenase kinase (PDK) inhibitor which can switch cell metabolism from glycolysis to oxidative phosphorylation (OXPHOS) (Stacpoole, 1989), was used in combined with EGFR-TKis, erlotinib or gefitinib, to elicit anti-cancer synergy in NSCLC cell lines with EGFR mutation (Yang and Tam, 2016). Although DCA has been applied in clinical practice for treating various disorders, such as diabetes, lactic acidosis as well as cancers, the chronic administration of DCA may produce toxicities, like peripheral neuropathy, which may be due to the pharmacokinetics alteration after repeated dosing (James et al., 2017). To pursue a more efficient strategy for combination treatment, we seek to apply dichloroacetophenone (DAP), a more potent PDH modulator in suppressing acute myeloid leukemia (AML) cell growth (Qin et al., 2016), in combination with EGFR inhibitors to explore any possible anti-cancer synergy in NSCLCs therapy. The anti-cancer mechanisms of this combination therapy will be elucidated.

2. Materials and methods

2.1. Cell lines and reagents

Human NSCLC cell lines, NCI-H1975, NCI-H1650 and A549 were obtained from ATCC, while NCI-H460 was a kind gift from Prof. Thomas Y.C. Leung (Department of Applied Biology and Chemical Technology, Faculty of Applied Science and Textiles, The Hong Kong Polytechnic University). All cell lines, except A549 which was maintained in DMEM (Gibco), were cultured in RPMI 1640 (Gibco). Media was supplied with 10% (v/v) Fetal Bovine Serum (Gibco, South America origin) and 1% (v/v) penicillin/streptomycin (Gibco), whereas cells were grown in a 5% CO_2 incubator at 37 ℃.

Erlotinib, gefitinib and Z-VAD-FMK were purchased from SelleckChem, while DCA and DAP were obtained from Sigma. All compounds were initially dissolved in DMSO (Sigma) as stock solutions, which were then diluted into whole media containing 0.1% DMSO. Primary and Secondary antibodies were all purchased from Cell Signaling Tech., except pyruvate dehydrogenase (PDH) and p-PDH which were obtained from Abcam, as well as Tubulin which was purchased from Invitrogen. Other reagents, such as MTT, crystal violet, etoposide, *etc.,* were obtained from Sigma.

2.2. MTT cell viability assay

Cell viability was detected using MTT assay. Cells were seeded in 96-well plates for 24 h before compounds of desired concentrations, either in mono-application or in combination, were loaded into specific well and cells were cultured for another 24, 48 or 72 h, respectively, depending on different study designs. Then, the supernatant of each well were removed and 100 µl media containing 0.5 mg/ml MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazoliumbromide) were loaded for 4 h. After incubation, the solvent was discarded, and O.D. value of each well was measured at 570 nm by SpectraMax M5 Microplate Reader (Molecular Devices) after formazan crystals were fully dissolved in 100 µl DMSO.

2.3. Colony formation assay

Colony formation assay were applied to compare the anti-cancer effects of different treatments. Cells were seeded in 6-well plates with specific cell density for 24 h, after which media with either compound alone or in combination was loaded for a continuous 3 days. After treatment, cells were refreshed with compound-free media every 3 days until 16 days after cell seeding. Then cell colonies were fixed with 95% ethanol followed by 0.1% crystal violet staining. Colonies with over 100 cells were defined as positive.

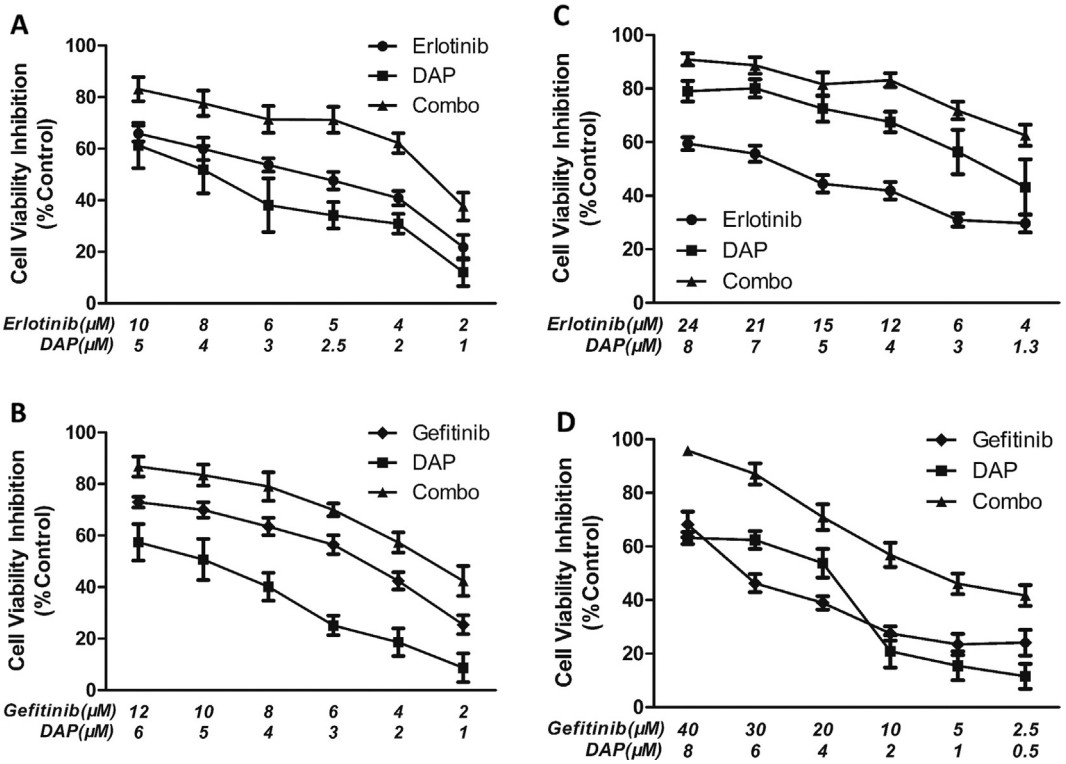

Fig. 1. DAP combination synergistically inhibited NCI-H1975 and NCI-H1650 growth. For MTT assay, NCI-H1975 (**A** and **B**) or NCI-H1650 (**C** and **D**) cells were seeded in 96-well plates, while varied concentration of DAP and erlotinib/gefitinib were dissolved simultaneously in whole media and loaded into individual wells for 72 h. CI value (**E**) of each combination in accordance with cell viability that affected by individual combined concentration of two compounds (fraction affected, Fa) was determined by the software Biosoft CalcuSyn. For colony formation assay (**F**), cells were seeded in 6-well plates and treated with specific treatments for 72 h. Media were refreshed every 3 days after the treatment, while number of colony clusters with over 100 cells were counted 16 days after cell seeding in either NCI-H1975 (**G**) or NCI-H1650 (**H**). * represented statistical significance between groups.