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Pculin02H, a curcumin derivative, inhibits proliferation and clinical drug resistance of HER2-overexpressing cancer cells

Jin-Cherng Lien [a], Chao-Ming Hung [b,c], Yi-Jing Lin [d], Hui-Chang Lin [a], Ting-Chia Ko [e], Li-Chung Tseng [f], Sheng-Chu Kuo [a], Chi-Tang Ho [g], Jang-Chang Lee [a,1], Tzong-Der Way [d,h,i,*]

[a] School of Pharmacy, College of Pharmacy, China Medical University, Taichung, Taiwan
[b] Department of General Surgery, E-Da Hospital, I-Shou University, Kaohsiung, Taiwan
[c] School of Medicine, I-Shou University, Kaohsiung, Taiwan
[d] Department of Biological Science and Technology, College of Life Sciences, China Medical University, Taichung, Taiwan
[e] Department of Pharmacy, Mackay Memorial Hospital, Hsinchu, Taiwan
[f] Department of Counseling Psychology, Toko University, Chiayi, Taiwan
[g] Department of Food Science, Rutgers University, New Brunswick, NJ, USA
[h] Institute of Biochemistry, College of Life Science, National Chung Hsing University, Taichung, Taiwan
[i] Department of Health and Nutrition Biotechnology, College of Health Science, Asia University, Taichung, Taiwan

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ABSTRACT

Amplification of the *HER2* gene (also known as *neu* or *ErbB2*) or overexpression of HER2 protein has become a solicitous therapeutic target in metastatic and clinical drug-resistance cancer. In our present work, a new series of curcumin derivatives were designed and synthesized using curcumin as model. Here, we evaluated whether curcumin derivatives have better efficiency to degrade HER2 than curcumin. Among these test compounds, pculin02H had better efficiency to inhibit the expression of HER2 than curcumin. Moreover, pculin02H preferentially suppressed the growth of HER2-overexpressing cancer cell lines. Pculin02H induced G2/M cell cycle arrest followed by apoptosis. Interestingly, our results suggested that a posttranslational mechanism contributed to pculin02H-induced HER2 depletion in HER2-overexpressing cancer cells. We found that pculin02H significantly enhanced the antitumor efficacy of clinical drugs on HER2-overexpressing cancer cells as well as efficiently reduced HER2-induced drug resistance. These findings may provide an alternative preventive or therapeutic strategy against HER2-overexpressing cancer cells.

1. Introduction

The structural modification from natural products to achieve more potent agents has historically been a mainstay source of anticancer drugs. These agents have been named smart drugs, and offer a rich source of compounds that have widespread applications in medicine. Plant derived natural compounds always fascinate scientists of being biocompatible, potentially safe and effective anticancer agents [1].

Numerous dietary chemopreventive compounds have shown potential in treating cancer. Curcumin, a polyphenol obtained from *Curcuma longa*, has been shown to display chemotherapeutic and chemopreventive effects in diverse cancers [2–4]. A series of curcumin derivatives have been designed and synthesized, a monocarbonyl analog of curcumin B63 has a greater anti-cancer capacity than the parent curcumin in colon cancer cells. The necrotic and apoptotic effects of B63 are mediated by ROS resulting from ER stress and mitochondrial dysfunction. 50 mg/kg of B63 inhibited tumor growth similarly to 100 mg/kg of curcumin in vivo [5]. A potent and irreversible cytotoxic activity of curcumin derivative bis-DeHydroxyCurcumin (bDHC) has been shown to induce autophagy on human colon cancer cells, but not on human normal cells. Moreover, ER-stress induced autophagy has a major role in

Abbreviations: CHX, cycloheximide; EGFR, epithelial growth factor receptor; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; NSCLC, non-small cell lung cancer; PBS, phosphate-buffered saline; PI, propidium iodide.

* Corresponding author at: Department of Biological Science and Technology, College of Life Sciences, China Medical University, No. 91 Hsueh-Shih Road, Taichung 40402, Taiwan. Tel.: +886 4 2205 3366x5209; fax: +886 4 2203 1075.
E-mail addresses: leejh@mail.cmu.edu.tw (J.-C. Lee), tdway@mail.cmu.edu.tw (T.-D. Way).
[1] Co-corresponding author. School of Pharmacy, College of Pharmacy, China Medical University, No. 91 Hsueh-Shih Road, Taichung 40402, Taiwan. Tel.: +886 4 2205 3366x5125; fax: +886 4 22030760.

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triggering bDHC-cell death [6]. Hydrazinobenzoylcurcumin (HBC) exhibits potent inhibitory activities against the proliferation of several tumor cells. It induces A549 cell autophagy and inhibits the viability of A549 cells [7]. (4-[3,5-bis-[2-(4-hydroxy-3-methoxyphenyl)-ethyl]-4,5-dihydropyrazol-1-yl]-benzoic acid), a curcumin derivative, inhibits the cell cycle progression of colon cancer cells via antagonizing Ca^{2+}/CaM functions [8]. Thus, synthetic curcumin derivatives display chemotherapeutic and chemopreventive effects via various mechanisms.

HER2 is a 185 kDa transmembrane receptor with tyrosine kinase activity. Although there is no known ligand that binds to HER2, the extracellular domain of HER2 remains in a constitutively active conformation, which makes HER2 a preferred partner for dimerization with other epithelial growth factor receptor (EGFR) family members, including EGFR, HER3, and HER4 [9]. Activation of the HER family receptors leads to phosphorylation of specific tyrosine residues, and recruitment of several proteins to the receptors resulting in activation of signaling pathways. The HER family members are able to activate different intracellular signaling cascades, including the phosphatidylinositol 3-kinase (PI3K)/Akt, the mitogen-activated protein kinase (MAPK), and the signal transducer and activator of transcription (STAT) pathways. It has been shown that these signaling proteins regulate cell proliferation and survival [9].

HER2 aberrations (gene amplification, gene mutations, and protein overexpression) have been frequently found in various types of human cancers. HER2 overexpression has now been described in a variety of tumors including breast cancer, gastric cancer, non-small cell lung cancer (NSCLC), ovarian cancer, salivary duct carcinoma, and pancreatic cancer [10]. The tumorigenic potential of HER2 in cancer cells has been proved to enhance cell proliferation, increase tendency for metastasis, shorten disease-free survival, drug-resistance, and lower overall survival rates [11]. For example, approximately 30% of breast cancer patients have overexpression of HER-2 and the overexpression metastasizes faster, induces resistance of the chemotherapy and down-regulates function of estrogen receptor. Trastuzumab (Herceptin), a recombinant humanized monoclonal antibody, inhibits proliferation of HER-2 overexpressing tumor cells and the use of that in combination with chemotherapeutic agents in metastatic breast cancer have increased cytotoxicity of chemotherapeutic agents [12].

Previous study found that curcumin and its derivatives effectively inhibits HER2 tyrosine kinase activity by depleting the expression of HER2 [13–17]. In the present study, thirty-two curcumin derivatives were chosen for evaluation of their effect on HER2 expression. Among them, pculin02H seemed to be more sensitive to inhibit the expression of HER2 than curcumin. Thus, among the tested curcumin derivatives, pculin02H is considered the most promising compound for further study of physiochemical properties and biological mechanisms.

2. Materials and methods

2.1. Chemicals and reagents

The analogs of curcumin were synthesized in our laboratory. Cell culture materials were obtained from Invitrogen (Burlington, Ontario, Canada). Antibodies and reagents were purchased from commercial sources: an antibody against HER2 (Ab3) was from Calbiochem company (San Diego, CA, USA); anti-mouse and anti-rabbit antibodies conjugated to horseradish peroxidase, a β-actin antibody 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT), actinomycin D, cycloheximide, G418, LY294002, PD98059, etoposide (E1383), taxol (T7402), and doxorubicin (D1515) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Primary antibodies phospho-ERK (T202/Y204), phospho-Akt (S473), ERK, and Akt were purchased from Cell Signaling Technology (Beverly, MA, USA).

2.2. Synthesis of curcumin derivatives

To a solution of boric oxide (350 mg, 5.0 mmol) in DMF (1.0 mL) was added acetylacetone (0.51 mL, 5.0 mmol), followed by tributyl borate (2.7 mL, 10 mmol) at 65 °C and stirred for 15 min. To the above borate complex, substituted benzaldehyde (10 mmol) was added and stirred for 5 min. A mixture of 1,2,3,4-tetrahydroquinoline (0.1 mL) and acetic acid (0.3 mL) in DMF (1 mL) was added to the reaction mixture and heated to 95 °C for 4 h. After cooling to 15 °C, acetic acid (20%, 50 mL) was added with stirring and again the reaction mixture was stirred at 70 °C for another 1 h. Then it was cooled to 15 °C, the so-formed solid was filtered, washed with water, and dried. The crude compounds **1**–**8** were chromatographed over silica gel column using chloroform/methanol (95:5) as eluent followed by recrystallisation from chloroform/methanol to give compounds **1**–**8**. And then the compounds **1**–**8** (0.1 mol) and hydrazine (or substituted hydrazine or hydroxylamine) (0.1 mol) were dissolved in 50 mL of ethanol in the presence of acetic acid (0.1 mol) and refluxed at 70 °C for 1 h. The resulting solution was concentrated and collected in a beaker. To this mixture, 20 mL of petroleum ether (40–60 °C) was added and kept at 0 °C for 48 h. A light brown solid mass obtained was collected and recrystallised in hot ethanol to give compounds **9**–**33**.

2.3. Cell culture

SKOV3 and SKBr3 cells, both of which overexpressing HER2, and MCF-7, MDA-MB-231 and MDA-MB 435/neo cells, all which expressing the basal level of HER2 were used in this study. Moreover, we used MCF-7/HER18 and MDA-MB 435/HER2; MCF-7 and MDA-MB 435 stably transfected with pSV2-*erbB2*. MDA-MB-231, and MCF-7 were grown in DMEM supplemented with 10% fetal bovine serum (FBS), and SKBr3 was cultured in MyCoy's 5A medium (modified). Other cells were cultured in DMEM/F12 supplemented with 10% FBS. Cells were grown in a humidified incubator at 37 °C under 5% CO_2 in air.

2.4. Western blot analysis

Western Blot was done as described previously [18]. Briefly, cells (2×10^6) were treated with various agents as indicated in figure legends. After treatment, cells were placed on ice, washed with cold phosphate-buffered saline (PBS), and lysed in the lysis buffer. Fifty micrograms of protein extract was loaded into sodium dodecyl sulfate–polyacrylamide gels, and the separated proteins were transferred to nitrocellulose filters. The filters were probed with the appropriate primary antibody.

2.5. Cell viability studies

The effects of curcumin, curcumin derivatives, and three clinical drugs (Doxorubicin, Etoposide, and Taxol) on cell viability were examined by MTT assay. Cells at a density of 1×10^4 cells per well were exposed to various agents for 24 h. MTT solution (stock concentration: 5 mg/mL in PBS) was diluted to 500 μg/mL. 100 μL of MTT working solution was treated to each well and incubated at 37 °C for 2 h. The optical density was measured using ELISA reader at 570 nm wavelength.