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Luteolin enhances paclitaxel-induced apoptosis in human breast cancer MDA-MB-231 cells by blocking STAT3

Mon-Yuan Yang [a], Chau-Jong Wang [a,d,1], Nai-Fang Chen [b], Wen-Hsin Ho [c], Fung-Jou Lu [b], Tsui-Hwa Tseng [b,d,*]

[a] Institute of Biochemistry and Biotechnology, Chung Shan Medical University, No. 110, Section 1, Chien-Kuo N. Road, Taichung, Taiwan
[b] School of Applied Chemistry, Chung Shan Medical University, No. 110, Section 1, Chien-Kuo N. Road, Taichung 402, Taiwan
[c] Department of Anatomy, School of Medicine, Chung Shan Medical University, No. 110, Section 1, Chien-Kuo N. Road, Taichung, Taiwan
[d] Department of Medical Education, Chung Shan Medical University Hospital, No. 110, Section 1, Chien-Kuo N. Road, Taichung 402, Taiwan

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ABSTRACT

The potential use of low-dose chemotherapy has been appealing because lower dosages are more attainable during cancer therapy and cause less toxicity in patients. Combination therapy of paclitaxel, a promising frontline chemotherapy agent, with natural anti-tumor agents that are considerably less toxic and possess the capability of activating additional apoptotic signals may provide a rational molecular basis for novel chemotherapeutic strategies. Luteolin, a natural flavone, possesses multiple biological activities, including anti-tumor potential. In the present study, the effects of concomitant administration of luteolin and paclitaxel were investigated in human breast cancer MDA-MB-231 cells. Luteolin alone demonstrated an anti-proliferative effect. Co-administration of luteolin and paclitaxel resulted in an increase in apoptosis compared with the treatment of paclitaxel alone as evidenced by the results of a diamidino-2-phenylindole (DAPI) stain and Annexin-V-based assay. Moreover, immunoblotting analysis also showed that the co-administration of luteolin and paclitaxel activated caspase-8 and caspase-3 and increased the expression of Fas. Furthermore, the increased expression of Fas due to co-administration was shown to be due to the blocking of signal transducer and activator of transcription 3 (STAT3). Finally, combination therapy with luteolin and paclitaxel significantly reduced tumor size and tumor weight in an orthotopic tumor model of MDA-MB-231 cells in nude mice. These results suggest that the luteolin–paclitaxel combination could be a novel strategy for the treatment of breast cancer.

Introduction

Paclitaxel (Taxol) is a naturally occurring antimitotic agent that is widely used as a cancer chemotherapeutic drug. Paclitaxel exhibits clinical activity in a range of human malignancies, including breast cancer [1]. Paclitaxel strongly binds to the N-terminal region of the β-subunit of tubulin and promotes the formation of highly stable microtubules that resist depolymerization, thereby preventing active tumor cell division and arresting the cell cycle at the G2/M phase. In addition, paclitaxel triggers apoptosis by both caspase-dependent and caspase-independent pathways [2]. Because paclitaxel also inhibits division of normal cells, the major limitation of paclitaxel is its extreme toxicity [3]. To overcome the problem, it has been suggested that modulation of tumor-specific signaling pathways may provide a different and complementary approach to the clinical management of the disease [4]. In addition, studies of combination chemotherapy have been focused on identifying natural compounds that could increase the therapeutic index [5,6].

Apoptosis, which is deregulated in many cancers, may be a primary mechanism of antineoplastic agents. Apoptosis is mediated through two major pathways, the extrinsic and intrinsic pathways, and both lead to the activation of caspases. The extrinsic pathway is triggered at the plasma membrane by the activation of death receptors, such as Fas, and subsequent activation of caspase-8, which activates downstream effector caspases such as caspase-3.

Abbreviations: DAPI, diamidino-2-phenylindole; MAPK, mitogen activated protein kinase; PARP, poly (ADP-ribose) polymerase; STAT3, signal transducer and activator of transcription 3.

* Corresponding author at: School of Applied Chemistry, Chung Shan Medical University, No. 110, Section 1, Chien-Kuo N. Road, Taichung 402, Taiwan. Tel.: +886 4 24730022; fax: +886 4 23248189.
E-mail address: tht@csmu.edu.tw (T.-H. Tseng).

[1] Equal contributed as the first author.

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Alternatively, the intrinsic pathway is triggered by various apoptotic stress signals and is characterized by mitochondrial dysfunction and activation of caspase-9 and caspase-3. The caspase proteases are synthesized as proenzymes that are cleaved into active heterodimers during cellular apoptosis [7,8]. Furthermore, the signal transducer and activator of transcription 3 (STAT3) is constitutively activated in many neoplastic cells. Activated STAT3 may control the expression of anti-apoptotic and pro-proliferative response genes [9]. In addition, the mitogen activated protein kinase (MAPK) pathway is activated by various extracellular signals to regulate the downstream phosphorylation of target proteins, including transcription factors and protein kinases involved in cell proliferation and apoptosis [10]. Therefore, perturbations of the STAT3 or MAPK pathways may regulate chemotherapy-mediated cancer cell death.

Luteolin, or 3′,4′,5,7-tetra-hydroxyflavone, is part of the flavones subclass of flavonoids that exist in many types of plants, including fruits, vegetables and medicinal herbs. Luteolin has multiple biological properties including the potential for cancer prevention and therapy [11]. Our previous data indicated that luteolin could induce apoptosis via JNK activation-mediated mitochondrial damage in HepG2 cells [12]. In addition, luteolin inhibits HGF-mediated migration and invasion of HepG2 cells by regulating the MAPK/ERK and the PI3K/AKT pathways [13]. In the present study, we investigated the effect and mechanism of co-administration of luteolin and paclitaxel in MDA-MB-231 breast cancer cells. The aim of this study was to provide a rational molecular basis for novel chemotherapeutic strategies.

Materials and methods

Chemicals

Dulbecco's modified Eagle's medium (DMEM), phosphate-buffered saline (PBS), fetal bovine serum, L-glutamine, penicillin/streptomycin (PS), and trypsin–EDTA were purchased from GIBCO Ltd., (Grand Island, NY, USA). Anti-phospho-ERK (Thr202/Tyr204), anti-phospho-JNK (Thr183/Tyr185), anti-phospho-p38 (Thr180/Tyr182), anti-phospho-STAT3 (Tyr705), anti-phospho-IκB-α (Ser 32) and anti-poly (ADP-ribose) polymerase (PARP) antibodies were purchased from Cell Signaling Technology (Beverly, MA). Other antibodies, such as anti-Bcl-2, anti-Bax, anti-Bcl-xl, anti-ERK1/2, anti-JNK, anti-p38 and anti-IκB-α antibodies, were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Luteolin, paclitaxel, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) and 4',6' -diamidino-2-pheneylindole (DAPI) were purchased from Sigma–Aldrich.

Cell culture

The estrogen-independent breast cancer cell lines MDA-MB-231 (ER^-, $HER2/neu^-$) and MDA-MB-453 (ER^-, $HER2/neu^+$) are metastatic breast cancer cell lines. The cells were maintained in DMEM supplemented with 10% fetal bovine serum, 1% L-glutamine and penicillin/streptomycin. The cells were cultured in an incubator with 5% CO_2 at 37 °C.

Cell growth and cell viability assay

Cell growth was assessed using a trypan blue dye exclusion assay. In brief, cells (2×10^4 cells/well) were seeded in 6-cm dishes. After treatment with different concentrations of luteolin for 24 h or 48 h, the cells were trypsinized and the viable cells were counted by trypan blue dye exclusion using a light microscope. Cell viability was determined using an MTT assay. In brief, cells (2×10^4 cells/well) were seeded in 24-well culture plates and exposed to different concentrations of luteolin, paclitaxel and combinations for 24 and 48 h. Next, the medium was changed and the cells were incubated with MTT (5 mg/ml) for 4 h. Finally, the absorbance of the formazan product was measured at a wavelength of 570 nm on an ELISA reader.

DAPI staining

Cells (2×10^5 cells/well) were grown in 6-well culture plates until adherent. After treatment with luteolin, paclitaxel and combinations, each well was washed with PBS and fixed with 3.7% formaldehyde for 30 min at room temperature. The fixed cells were permeabilized by 0.1% Triton X-100 for 10 min, the supernatant was removed, and the cells were washed three times with PBS. The cells were incubated with DAPI (5 μM) for 1 h at room temperature in the dark. DAPI-stained cell nuclei (apoptotic nuclei stained intensely) were observed with an inverted fluorescence microscope.

Flow cytometric detection of apoptosis by Annexin-V-FITC and propidium iodide staining

A combination of Annexin-V-FITC and propidium iodide staining allows for distinction between early ($annexin^+/PI^-$) and late ($annexin^+/PI^+$) apoptotic cells, necrotic cells and live cells. After cells were treated with luteolin, paclitaxel and combinations, the cells were trypsinized and washed with PBS and resuspended in 100 μl binding buffer containing 5 μl Annexin-V-FITC and/or 5 μl propidium iodide (Becton Dickinson, Annexin-V-FITC Apoptosis Detection Kit I). The cells were incubated for 15 min at room temperature in the dark. The cells were immediately analyzed by flow cytometry (Becton Dickinson, FACS Calibur). The percentages of apoptotic cells (Annexin-V positive cells) are presented as the mean ± SD.

Preparation of total cell extracts and immunoblot analysis

Cells (1×10^6 cells/well) were seeded in 10-cm dishes in the presence of the luteolin, paclitaxel and combinations. The cells were collected by trypsin–EDTA and lysed in RIPA buffer (50 mM Tris–HCl, 1 mM EDTA, 150 mM NaCl, 1% NP-40) containing protease inhibitors. After mixing for 30 min at 4 °C, the mixtures were centrifuged (10,000×*g*) for 10 min at 4 °C and the supernatants were collected as whole-cell extracts. The protein content was determined using the Bio-Rad protein assay reagent and bovine serum albumin as a standard. An equal amount of protein from the total cell extracts was boiled for 8 min. The extracts were

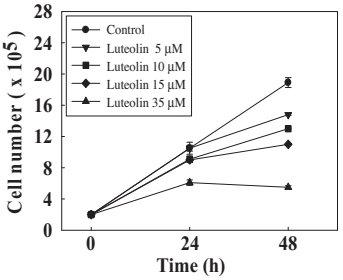

Fig. 1. The effect of luteolin on cell growth in MDA-MB-231 cells. Cells were treated with the indicated concentrations of luteolin for 24 and 48 h. The viable cells were determined using trypan blue dye exclusion assays. Data are presented as means ± SD of three independent experiments.