Cellular & Molecular Biology
Cell. Mol. Biol. 2015; 61 (7): 123-127
Published online November 30, 2015 (http://www.cellmolbiol.com)
Received on June 28, 2015, Accepted on November 26, 2015.
doi : 10.14715/cmb/2015.61.7.19

Wogonin increases doxorubicin sensitivity by down-regulation of IGF-1R/AKT signaling pathway in human breast cancer

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Abstract

Currently drug resistance has remained a major challenge in successful breast cancer therapy. Wogonin, one of the active components of *scutellaria baicalensis*, has shown anticarcinogenic, chemopreventive, and immunoregulatory functions. The present study aimed to explore whether wogonin exerted synergistic cytotoxicity with doxorubicin in breast cancer. Our data indicated that wogonin inhibited the proliferation of breast cancer cells in a dose- and time-dependent manner. Combined treatment with wogonin increased the doxorubicin sensitivity in breast cancer cells. Moreover, administration with wogonin alone or in combination with doxorubicin suppressed the expression of insulin like growth factor 1 receptor (IGF-1R) in Bcap-37 and MCF-7 cells. Incubation with insulin like growth factor (IGF) I or IGF-II promoted cell growth, which was reversed by wogonin co-administration. Mechanically, we found that down-regulation of IGF-1R diminished the chemosensitization role of wogonin in breast cancer. In addition, wogonin suppressed the phosphorylation levels of AKT and addition of AKT inhibitor abolished the synergistic cytotoxicity of wogonin and doxorubicin. Taken together, combined treatment with wogonin increased the doxorubicin sensitivity in breast cancer cells through regulation of IGF-1R/AKT signaling pathway. Therefore, these findings demonstrated that combination therapy with wogonin led to better therapeutic effects via regulating IGF-1R/AKT signaling pathway in doxorubicin-based chemotherapy for breast cancer.

Key words: Breast cancer, wogonin, combined therapy, IGF-1R, AKT.

Introduction

Breast cancer is one of the most common malignancies in females worldwide, and. it has become the leading cause of cancer-related deaths among women (1). At present, combination chemotherapy serves as one of the important adjuvant therapies for breast cancer after surgery in the clinic (2). Despite the significant improvements of surgical operation and chemotherapy regimens, relapse remains a major challenge in patients with advanced breast cancer (3). Thus, it is important to develop novel and effect drugs to reverse the resistance to traditional therapeutics.

Scutellaria baicalensis Georgi has been extensively used as a medicinal herb for its purported ability to treat allergic reactions, inflammatory diseases, and tumors (4,5). Wogonin (5,7-dihydroxy-8-methox flavone) is one of the active components of *scutellaria baicalensis*, exhibiting anticarcinogenic, chemopreventive, and immunoregulatory properties (6,7). Accumulating evidences have demonstrated that wogonin treatment significantly suppresses the proliferation, invasion and migration and induces cell cycle arrest and apoptosis in several cancers such as melanoma, hepatocellular carcinoma, breast cancer, colorectal carcinoma (8-10). In addition, multiple studies have suggested that the molecular mechanisms of wogonin's anti-cancer effects are mainly attributed to the anti-oxidant activity, apoptosis activation, and anti-angiogenesis activity (11). However, whether wogonin can sensitize tumor cells to traditional chemotherapeutics has not been revealed. In the current study, we aimed to explore the chemotherapeutic sensitization and underlying mechanism of wogonin in breast cancer cells treated with doxorubicin.

Materials and methods

Cell culture and reagents

Human breast cancer cell lines MCF-7 and Bcap-37 were purchase d from the ATCC (Manassas, VA, USA) and cultured in DMEM (Gibco, Carlsbad, CA, USA) supplemented with 10% FBS and 1% penicillin⁄streptomycin. All cells were maintained at 37°C in 5% CO_2 incubator. Doxorubicin and wogonin were purchased from Sigma-Aldrich (St. Louis, MO, USA). The IGF-1R siRNA and negative control siRNA were purchased from Santa Cruz Biotechno logy (Santa Cruz, CA, USA).

Cell viability assay

Cells at the density of 3000 cells⁄well were seeded onto 96-well plates and incubated with different concentrations of drugs for indicated time points. Then 10 μL ⁄well CCK8 solution (Dojindo, Kumamoto, Japan) was added, the plates incubated for 3 h, and absorbance was measured at 450 nm using an MRX II microplate reader (Dynex, Chantilly , VA, USA).

EdU incorporation assay

Cell viability was calculated as a percentage of

untreated control. Measurement of inhibitive rate of cell proliferation was carried out using a Click-iT EdU Imaging Kit (Invitrogen, Carlsbad , CA, USA) following the manufacturer's instruction.

Western blot analysis

Cultured cells were lysed in 50 µl cell lysis buffer (Cell Signaling, Danvers, MA , USA) and protein concentration was quantified using the BCA Protein Kit (Thermo, Rockford, IL, USA). Cell lysates were subjected to SDS-PAGE and then proteins were transferred to polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA). The membranes were incubated with primary antibodies (IGF-1R and IGFBP-3; Abcam, Cambridge, USA) followed by horseradish peroxidase-conjugated secondary antibody (Abcam, USA). Protein expression was detected by chemiluminescence (GE Healthcare, Piscataway, NJ, USA).

Statistical Analysis

All data are presented as mean±SD. For comparisons between two different groups, statistical significance was assessed using the Student's t-test. Comparisons among groups were performed using analysis of variance (ANOVA). $P<0.05$ was considered to indicate statistically significant differences.

Results

Wogonin increased doxorubicin cytotoxicity in breast cancer cells

Firstly, CCK-8 assay was conducted to assess the inhibitory effects of wogonin on breast cancer cell growth. Two breast cancer cell lines Bcap-37 and MCF-7 were incubated with wogonin at different concentrations for 24, 48, and 72 h. Results showed that wogonin inhibited the proliferation of Bcap-37 and MCF-7 (Fig. 1A) in a dose- and time-dependent manner. Interestingly, our results indicated that combined treatment with wogonin increased the sensitivity of Bcap-37 and MCF-7 to doxorubicin as shown by CCK-8 assay (Fig. 1B) and EDU incorporation assay (Fig. 1C).

Wogonin co-administration inhibited the expression of IGF-1R in breast cancer cells

We then performed western blot to measure the expression of IGF-1R and IGFBP-3 in breast cancer cells treated with wogonin at different doses. Results showed that wogonin treatment alone significantly suppressed the IGF-1R expression and increased the protein level of IGFBP-3 in a concentration-dependent manner (Fig. 2A). In addition, IGF-1R expression was significantly down-regulated after combined treatment with wogonin and doxorubicin in Bcap-37 and MCF-7 cells (Fig. 2B).

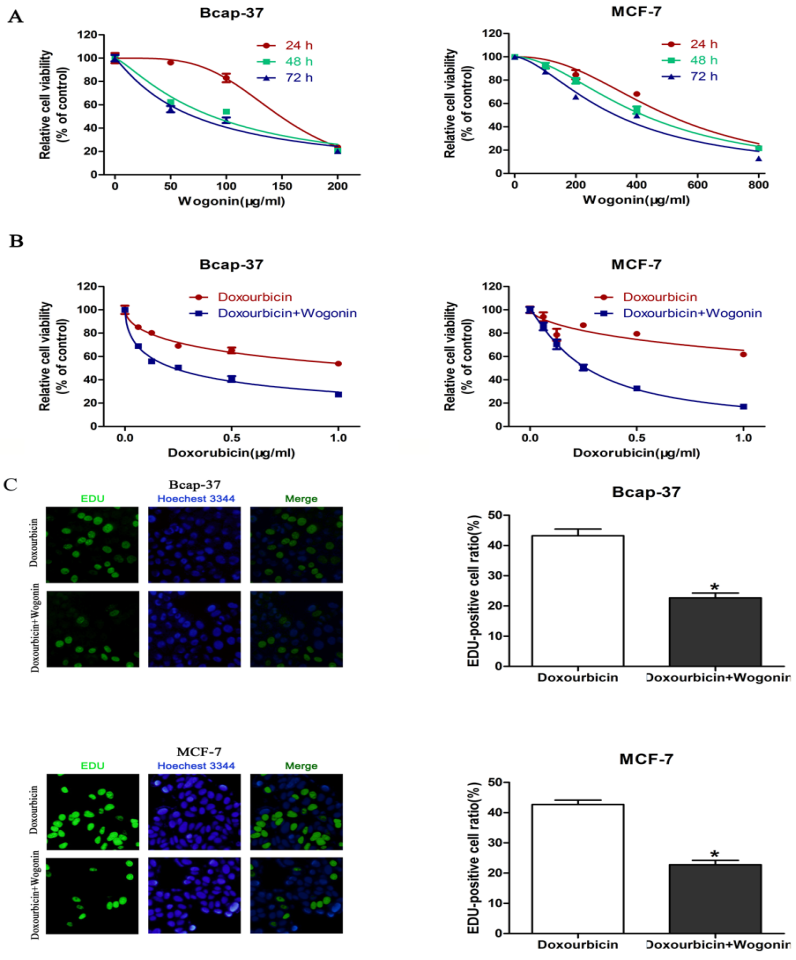

Figure 1. Wogonin increased doxorubicin cytotoxicity in breast cancer cells. Two breast cancer cells including Bcap-37 and MCF-7 were exposed to wogonin (A) alone or in combination with doxorubicin (B) for indicated time points. CCK-8 assay was performed to determine cell viability. Photomicrographs and bar charts depict the EdU staining and relative EdU-positive ratio, respectively, of Bcap-37 and MCF-7 cell after treatment with doxorubicin or doxorubicin plus wogonin. * $P < 0.05$.

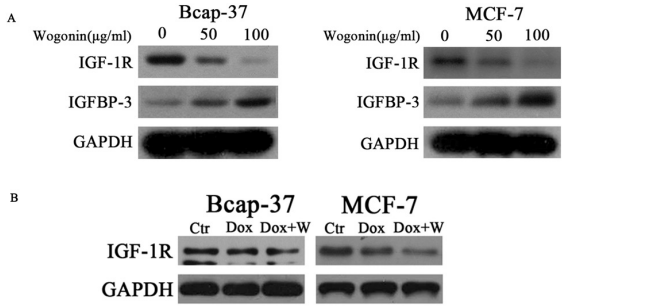

Figure 2. Wogonin co-administration inhibited IGF-1R expression in breast cancer cells. Western blot was conducted to measure the expression of IGF-1R and IGFBP-3 in Bcap-37 (A) and MCF-7 (B) cells treated with wogonin at different doses. (C) Determination of IGF-1R in Bcap-37 and MCF-7 cells treated with doxorubicin plus wogonin.

Wogonin sensitized breast cancer cells to doxorubicin via inhibiting IGF-1R

Breast cancer cells were incubated with IGF-I or IGF-II alone or in combination at different doses for indicated time. CCK-8 assay showed that the cell viabilities of Bcap-37 (Fig. 3A) and MCF-7 (Fig. 3B) were remarkably elevated after treatment with IGF-I or IGF-II alone or in combination. However, combined treatment with wogonin blocked the proliferative roles of IGF-I and IGF-II in breast cancer cells (Fig. 3C). Furthermore, IGF-1R siRNA was transfected into breast cancer cells to down-regulate IGF-1R expression. Western blot analysis showed that the protein expression of IGF-1R was obviously decreased in breast cancer cells (Fig. 4E). As a result, down-regulation of IGF-1R increased the doxorubicin cytotoxicity in Bcap-37 (Fig. 4A) and MCF-7 (Fig. 4B). On the contrary, wogonin had little effects on IGF-1R siRNA- transfected breast cancer cells exposed to doxorubicin (Fig. 4C and D). In addition, IGF-1R siRNA- transfected cells exhibited lower sensitivity to

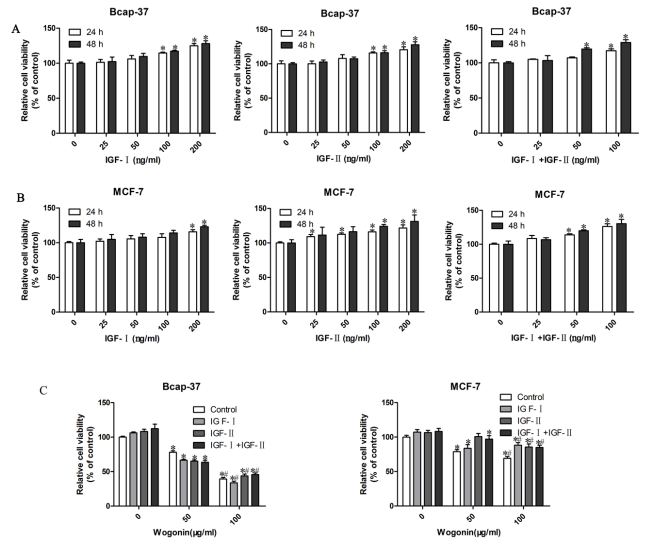

Figure 3. Wogonin blocked the proliferative roles of IGF-I and IGF-II. Breast cancer cells including Bcap-37 (A) and MCF-7 (B) were incubated with IGF-I or IGF-II alone or in combination at different doses for indicated time, and cell viabilities were assessed by CCK-8 assay. (C) Determination of cell viability in Bcap-37 and MCF-7 cells treated with IGF-I or IGF-II alone or in combination in the presence of wogonin.

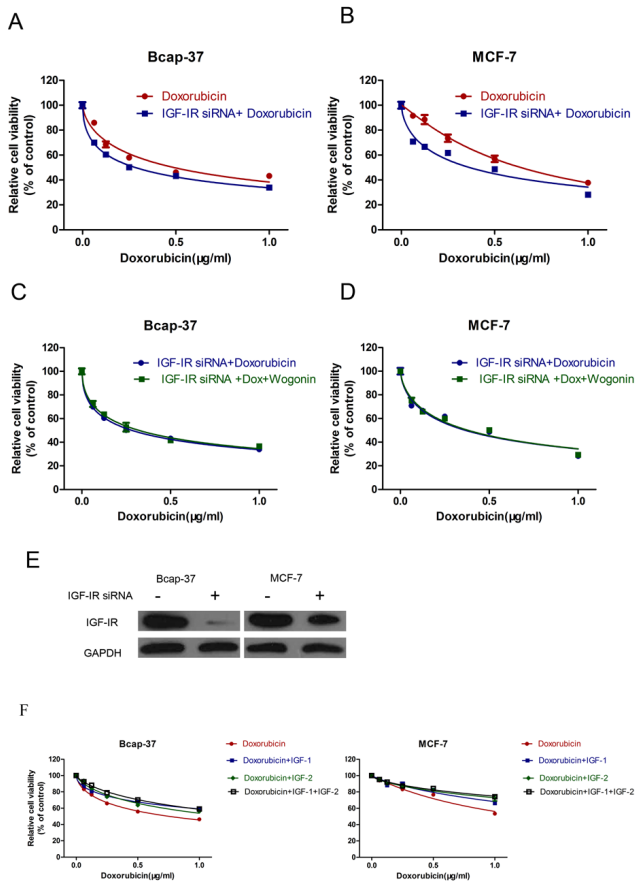

Figure 4. Wogonin sensitized breast cancer cells to doxorubicin via inhibiting IGF-1R. Bcap-37 (A) and MCF-7 (B) transfected with IGF-1R siRNA exhibited increased sensitivity to doxorubicin. Down-regulation of IGF-1R abolished the inhibitory effects of wogonin on Bcap-37 (C) and MCF-7 (D) cell viability exposed to doxorubicin. (E) Determination of IGF-1R expression by western blot. Decreased IGF-1R inhibited doxorubicin cytotoxicity in Bcap-37 and MCF-7 cells (F).

doxorubicin after addition of IGF-I or IGF-II alone or in combination (Fig. 4F).

Wogonin inhibited the activity of AKT signaling pathway in breast cancer cells

Furthermore, we examined the effects of wogonin on AKT signaling pathway. Western blot analysis indicated that combined administration with wogonin and doxorubicin reduced the phosphorylation level of AKT in Bcap-37 and MCF-7 cells (Fig. 5A). Furthermore, incubation with AKT inhibitor GSK690693 abolished the synergistic cytotoxicity of wogonin (Fig. 5B). Examination of Erk1/2 and AKT activities indicated that wogonin treatment inhibited IGF-I and IGF-II induced-phosphorylation of AKT in Bcap-37 and MCF-7 cells (Fig. 5C).

Discussion

The objectives of developing Chinese medicinal herbs are to reduce the traditional drug-related side effects and to increase the sensitivity of chemotherapeutics on tumor cells. Wogonin has been shown to possess several biological functions including anti-inflammation, anti-oxidant, especially in anti-tumor functions (12). Previous studies suggested that wogonin could induce

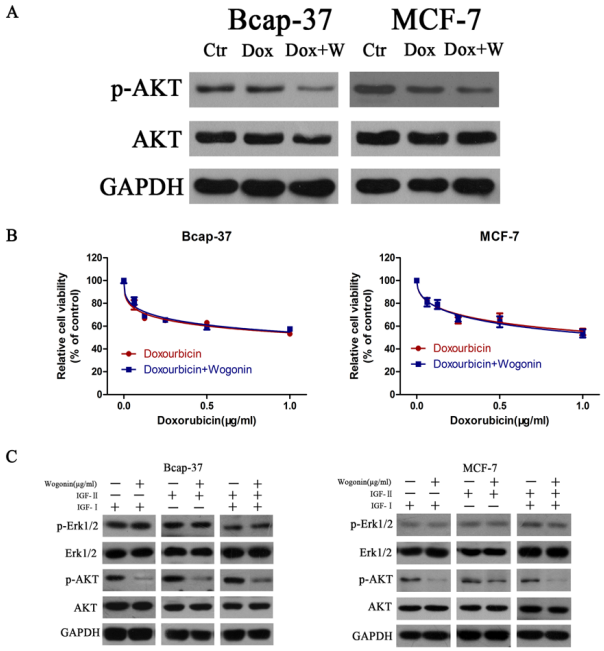

Figure 5. Wogonin inhibited the activity of AKT signaling pathway in breast cancer cells. (A) Western blot was performed to measure the phosphorylation level of AKT in Bcap-37 and MCF-7 cells exposed to doxorubicin alone or in combination with wogonin. (B) Assessment of cell viability after addition of AKT inhibitor. (C) Erk1/2 and AKT activities Bcap-37 and MCF-7 cells exposed to wogonin alone or combined with IGF-I and IGF-II.

apoptosis and inhibit invasion and migration in cancer cells (13). In the present work, we reported that wogonin increased the doxorubicin cytotoxicity in breast cancer cells through inhibition of IGF-1R.

Consistent with previous data, CCK-8 assay revealed that wogonin inhibited the proliferation of breast cancer cells in a dose- and time-dependent manner. In addition, our study showed that combined treatment with wogonin increased the doxorubicin sensitivity in breast cancer cells, suggesting the chemosensitization role of wogonin.

The insulin like growth factor 1 (IGF-1) system, composed of IGF-1, IGF-binding proteins (IGFBPs) and the IGF-1 receptor (IGF-1R), plays an important role in human physiology, especially in the development and function of mammary gland (14). In both normal mammary gland and malignant breast tissues, IGF-1R is mainly expressed by epithelial and only rarely by stromal cell (15). In addition, up-regulated IGF-1R levels have been detected in many cases of breast cancer, most often independently of cancer subtype, progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2), or estrogen receptor (ER) status (16). Increased IGF-1R levels strongly correlate with poor clinical outcomes across different molecular subtypes in breast cancer (17). Functional analysis has shown that IGF-1R involves in a wide range of cellular processes, such as proliferation, differentiation, apoptosis, DNA repair, protein synthesis, and oncogenesis (18,19). In this work, we found that wogonin treatment alone or in combination with doxorubicin significantly suppressed the protein expression of IGF-1R in tumor cells Moreover, our data showed that combined treatment with wogonin blocked the proliferative roles of IGF-I and IGF-II in breast cancer cells. In order to validate whether the anti-proliferative effects of wogonin on breast cancer .was mediated by IGF-1R, we decreased IGF-1R expression with siRNA technology. As a result, down-regulation of IGF-1R diminished the chemosensitization role of wogonin in breast cancer. These results demonstrated that wogonin sensitized breast cancer cells to doxorubicin via inhibition of IGF-1R.

It is well known that AKT signaling pathway plays a critical role in multiple biological activities, including proliferation, apoptosis, invasion and migration (20,21). Up-regulation of AKT is observed in several types of cancer, and it can be associated with uncontrolled cell growth (22,23). A recent study has reported that IGF-1 stimulation increases cell growth via activation of ERα, which consequently binds to IGF-1R, inducing downstream phosphorylation of AKT (24,25). Our study showed that wogonin treatment alone or in combination with doxorubicin suppressed IGF-I and IGF-II induced phosphorylation of AKT in Bcap-37 and MCF-7 cells. In addition, addition of AKT inhibitor abolished the synergistic cytotoxicity of wogonin and doxorubicin. Taken together, these results suggested the critical role of IGF-1R/AKT signaling in wogonin-regulated growth inhibition in breast cancer cells.

In conclusion, our present work demonstrated that combined treatment with wogonin increased the doxorubicin sensitivity in breast cancer cells through regulation of IGF-1R/AKT signaling pathway. Therefore, combination therapy with wogonin may contribute to a better therapeutic effect in doxorubicin-based chemotherapy for breast cancer.

Acknowledgements

This study was supported by Traditional Chinese medicine scientific research fund project of Zhejiang province (No. 2013ZA076; No.2012ZA079); Zhejiang province natural science fund (No. LY13H160006); co-constructed plan by Ministry of Health and Zhejiang Province (No. 201343278), and Zhejiang province natural science fund (No. LQ13H160006)".

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