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Magnolol down-regulates HER2 gene expression, leading to inhibition of HER2-mediated metastatic potential in ovarian cancer cells

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ABSTRACT

Overexpression of the HER2 oncogene contributes to tumor cell invasion, metastasis and angiogenesis and correlates with poor prognosis. Magnolol has been reported to exhibit anti-tumor activities. However, the molecular mechanism of action of magnolol has not been investigated in HER2-positive cancer cells. Therefore, we examined the anti-cancer effects of magnolol on HER2-overexpressing ovarian cancer cells. Magnolol treatment caused a dose-dependent inhibition of HER2 gene expression at the transcriptional level, potentially in part through suppression of NF-κB activation. Treatment of HER2-overexpressing ovarian cancer cells with magnolol down-regulated the HER2 downstream PI3K/Akt signaling pathway, and suppressed the expression of downstream target genes, vascular endothelial growth factor (VEGF), matrix metalloproteinase 2 (MMP2) and cyclin D1. Consistently, magnolol-mediated inhibition of MMP2 activity could be prevented by co-treatment with epidermal growth factor. Migration assays revealed that magnolol treatment markedly reduced the motility of HER2-overexpressing ovarian cancer cells. Furthermore, magnolol-induced apoptosis in HER2-overexpressing ovarian cancer cells was characterized by the up-regulation of cleaved poly(ADP-ribose) polymerase (PARP) and activated caspase 3. These findings suggest that magnolol may act against HER2 and its downstream PI3K/Akt/mTOR-signaling network, thus resulting in suppression of HER2-mediated transformation and metastatic potential in HER2-overexpressing ovarian cancers. These results provide a novel mechanism to explain the anti-cancer effect of magnolol.

Abbreviations: Akt, serine/threonine-specific protein kinase; β-gal, β-galactosidase; DMEM, Dulbecco's modified Eagle's medium; DMSO, dimethyl sulfoxide; EGF, epidermal growth factor; ERK, extracellular signal-regulated kinases; FBS, fetal bovine serum; GSK3β, glycogen synthase kinase 3β; MAPK, mitogen-activated protein kinase; MMPs, matrix metalloproteinases; mTOR, mammalian target of rapamycin; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; NF-κB, nuclear factor kappa B; p38MAPK, p38 mitogen-activated protein kinase; PARP, poly(ADP-ribose) polymerase; PBS, phosphate-buffered saline; PI3K, phosphoinositol 3-kinase; PLC, phospholipase C; PKC, protein kinase C; STAT, signal transducer and activator of transcription; VEGF, vascular endothelial growth factor.

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1. Introduction

Epithelial ovarian cancer is one of the major causes of cancer-related death among women worldwide. Between 1.8% and 35% of human epithelial ovarian cancer patients have been reported to overexpress HER2 (also known as ErbB2) [1]. The HER2 gene encodes a 185 kDa transmembrane tyrosine kinase receptor belonging to the epidermal growth factor receptor (EGFR) family. Aberrant up-regulation of HER2 is found in many cancer types and has been shown to promote cell survival, tumor growth, metastasis and angiogenesis [2,3]. Overexpression of HER2 in cancer patients is a marker of poor prognosis [4]. HER2 homo- or heterodimerizes with other members of EGFR family, and activates a variety of signaling pathways, including phosphoinositol 3-kinase (PI3K)/Akt and Ras/mitogen-activated protein kinase (MAPK), which control cell growth, motility, cell invasiveness, angiogenesis, adhesion and differentiation [4–6].Therefore, inhibition of HER2 expression in tumors could serve as a good approach for the development of novel cancer treatment.

Magnolol (5,5′-diallyl-2,2′-dihydroxybiphenyl), a main constituent of the root and stem bark of *Magnolia officinalis*, has been reported to exert multiple pharmacological effects, including anti-tumor [7–13], anti-inflammatory [14–16] and antimicrobial effects [15,17,18], and induces differentiation [19] and calcium mobilization [20]. Recent studies have demonstrated that magnolol can inhibit cell growth by blocking DNA synthesis and inducing apoptosis and cell cycle arrest in several cancer cell lines [7,9–13].

Although the anti-tumor pharmacological applications of magnolol have been documented, little is known about its effects and molecular mechanisms in HER2-overexpressing cancer cells. In this study, we investigate the anti-tumor effects of magnolol on HER2-overexpressing ovarian cancer cells. Our results show that magnolol significantly reduced the proliferative, clonogenic and metastatic potential of HER2-overexpressing SKOV3 ovarian cancer cells by down-regulating HER2 and its downstream PI3K/Akt-signaling pathway.

2. Materials and methods

2.1. Cells, chemicals and reagents

SKOV3 human ovarian cancer cells and BT474 human breast cancer cells were cultured in DMEM/F12 media (Invitrogen, CA, USA) supplemented with 10% heat-inactivated fetal bovine serum (FBS; Invitrogen). TOV21G human ovarian cancer cells were cultured in MCDB 105/Medium 199 (1:1, v/v) (Sigma–Aldrich, St. Louis, MO) media supplemented with 15% FBS. All of the cells were maintained in a humidified incubator with 5% CO_2/95% air at 37 °C. Magnolol (Wako, Osaka, Japan), was dissolved in DMSO and added to the cultured cells. Epidermal growth factor (EGF) and LY 294002 were purchased from Cell Signaling (MA, USA).

2.2. Proliferation and focus formation assay

Cells were seeded at 4000 per well in 96-well microtiter plates and incubated at 37 °C. Then cells were treated with different concentrations of magnolol or an equal volume of DMSO (0.1%, v/v) for 48 and 72 h. The cytotoxicity of magnolol was determined by the MTT metabolic assay as previously described [21]. To evaluate the long-term effects of magnolol on SKOV3 cells, the cells (100 per well) were treated with varying concentrations of magnolol for 6 h. After treatment, the cells were rinsed with fresh media and incubated in complete media for 12 days. The cells were then fixed and stained (0.02% crystal violet) for focus counting.

2.3. HER2 promoter activity assay

The day before transfection, SKOV3 cells were seeded in 6-well plates at a density of 4×10^5 cells per well. Using Arrest-In transfection reagents (Open Biosystems, USA), the cells were co-transfected with a luciferase reporter plasmid (pHER2-luc, containing approximately 1 kb of the human HER2 promoter fused to the luciferase reporter gene) and a pCMV-β-gal plasmid for 6 h, and then incubated in several concentrations of magnolol for 48 h. Analyses of luciferase reporter gene and β-galactosidase (β-gal) gene activities were performed as previously described [22]. The relative light units of luciferase activity were normalized against β-gal activity.

2.4. Reverse transcription-PCR (RT-PCR)

RNA extraction and cDNA preparation were performed as described [23] with slight modification. Briefly, total cellular RNA was prepared using TRIzol reagent (Invitrogen). Reverse transcription was carried out by oligo-d$(T)_{15}$ priming in the presence of M-MLV reverse transcriptase (Promega, WI, USA) at 37 °C for 1h. Polymerase chain reaction (PCR) was performed with primer pairs for HER2 (forward: 5′-AACTGCACCCACTCCTGTG-3′; reverse: 5′-TGA TGAGGATCCCAAAGACC-3′) and for β-actin internal control (forward: 5′-CTGTGGCATCCACGAAACTA-3′; reverse: 5′-CG CTCAGGAGGAGCAATG-3′). The PCR products were subjected to electrophoresis onto a 2.5% agarose gel, and were visualized by ethidium bromide staining.

2.5. Real-time quantitative RT-PCR (RT-qPCR)

Total RNA was isolated using TRIzol reagent (Invitrogen, CA, USA) from SKOV3 cells treated with or without magnolol according to the manufacturer's instructions. The concentration and purity of extracted RNA were measured by spectrophotometer. After RNA isolation, cDNA was formed after using reverse transcription kit (Promega, Madison, WI), as our previous report [23]. The ABI 7300 Real-Time PCR system (Applied Biosystems) was applied for the following quantitative real-time PCR (qPCR) with SYBR Green I as a double-strand DNA-specific binding dye and continuous fluorescence monitoring. The designed