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Original Article

Myricetin suppresses invasion and promotes cell death in human placental choriocarcinoma cells through induction of oxidative stress

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ABSTRACT

Myricetin is a bioactive compound found in a variety of vegetables and fruits, and its anti-cancer effects are well known. In this study, we confirmed that myricetin reduced proliferation of two choriocarcinoma cell lines (JAR and JEG-3) and also promoted apoptosis and regulated cell cycle progression in a dose-dependent manner in JAR and JEG-3 cells. In addition, we found that invasive and pro-angiogenic properties of malignant JAR and JEG-3 trophoblast cells were attenuated by myricetin treatment via MAPK and PI3K/AKT signaling pathways. In addition, we found that ROS production, lipid peroxidation, glutathione depletion, and loss of mitochondrial membrane potentials were enhanced in JAR and JEG-3 cells treated with myricetin. Moreover, myricetin augmented cytosolic Ca^{2+} release from the endoplasmic reticulum associated with modulation of ER stress in JAR and JEG-3 cells. Our results also revealed that myricetin had synergistic antiproliferative effects with current chemotherapeutics, etoposide and cisplatin, on choriocarcinoma cells. Collectively, results of the present study provide strong evidence for the potential of myricetin to be an effective therapeutic for the prevention of human placental choriocarcinomas.

Introduction

Choriocarcinomas are derived from chorionic epithelium and exhibit cancerous features including a high rate of vascular invasion that results in rapid hematogenous spread to various organs including lung, brain, and liver [1]. Therefore, suppression of metastasis or angiogenesis is a primary therapeutic strategy for treatment of choriocarcinomas by targeting placenta-derived growth factors or a variety of proteases present in the extracellular matrix [2]. Although cytotoxic agents including etoposide and cisplatin can be applied intravenously to treat choriocarcinomas, the mechanisms underlying tumorigenesis and metastasis of choriocarcinomas are not known [3].

Myricetin is a bioactive flavonol possessing anti-oxidant properties and it is derived from nuts, berries, and grapes. The anti-cancer effects of myricetin on several types of cancers including ovarian, gastric, and colon cancer have been reported [4]. The mechanisms inherent in the anti-cancer effects of myricetin are accompanied with a variety of changes within the cells such as double strand breakage of DNA, endoplasmic reticulum (ER) stress, and cell cycle arrest followed by apoptosis of cells. Despite its anti-proliferative, anti-angiogenic and anti-invasive functions in diverse cancer cells, the functional roles and underlying mechanisms of action of myricetin on human choriocarcinoma cells are unclear.

In the present study, we examined anti-cancer effects of myricetin with respect to viability, apoptosis, and invasion on two choriocarcinoma cell lines; JAR and JEG-3. Also, we identified underlying signal pathways in JAR and JEG-3 cells mediated by myricetin using pharmaceutical inhibitors.

Materials and methods

Chemicals

Myricetin was purchased from Sigma–Aldrich, Inc (St. Louis, MO, USA). U0126, SB203580, and SP600125 were from Enzo Life Science (Farmingdale, NY, USA) and LY294002 was from Cell Signaling Technology (Beverly, MA, USA). All antibodies were purchased from Cell Signaling Technology.

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Cell culture

JAR and JEG-3 cells were purchased from the American Type Culture Collection (Manassas, VA, USA) and maintained as described previously [5].

Proliferation assay

A cell proliferation ELISA, BrdU kit (Roche, Indianapolis, IN, USA) was used to determine effects of treatments on proliferation of JAR and JEG-3 cells according to the manufacturer's recommendations and as described previously [5].

Immunofluorescence microscopy

The effects of myricetin on the expression of proliferating cell nuclear antigen (PCNA) were determined by immunofluorescence microscopy as described previously [5]. JAR and JEG-3 cells were probed with mouse anti-human monoclonal antibody to PCNA (SantaCruz, CA, USA), and then incubated with goat anti-mouse IgG Alexa 488 (Invitrogen, Carlsbad, CA, USA).

Determination of apoptosis by annexin V and propidium iodide (PI) staining

The FITC Annexin V apoptosis detection kit I (BD Biosciences, Franklin Lakes, NJ, USA) was used to assess apoptosis of JAR and JEG-3 cells induced by myricetin as described previously [5].

TUNEL assay

Terminal deoxynucleotidyl transferase dUTP nick end-labeling (TUNEL) assay was used to assess effects of myricetin on of JAR and JEG-3 cells as described previously [5]. The cells were subjected to a TUNEL staining mixture using the *In Situ* Cell Death Detection kit, TMR red (Roche).

Cell cycle analysis

To examine the distribution of JAR and JEG-3 cells in the subG1, G1, S, and G2/M phases of the cell cycle, myricetin-treated cells were stained with propidium iodide (PI; BD Biosciences) in the presence of 100 μg/ml RNase A (Sigma). Fluorescence intensity was analyzed using a flow cytometer (BD Biosciences).

Transwell cell migration assay

Transwell cell migration assay was examined using 8-μm pore Transwell inserts (Corning, Inc., Corning, NY) as described previously [5].

VEGF enzyme-linked immunosorbent assay (ELISA)

A Quantikine human VEGF ELISA kit (R&D Systems, Minneapolis, MN, USA) was used to determine the abundance of vascular endothelial growth factor A (VEGFA). The conditioned medium was collected and concentrations of VEGFA determined by measuring the absorbance at 450 nm and 540 nm using an ELISA reader according to the manufacturer's instructions.

Quantitative RT-PCR analysis

Target gene expression was determined as described previously [5]. Primer sets are listed in Table 1.

Table 1
Primer sets used in quantitative RT-PCR.

Gene symbol	GenBank accession no.	Sense primer (5′ → 3′)	Antisense primer (5′ → 3′)
GAPDH	NM_001256799	GGCTCTCCAGAACATCATCC	TTTCTAGACGGCAGGTCAGG
VEGFA	NM_001025366	TTGTACAAGATCCGCAGACG	TCACATCTGCAAGTACGTTCG
Flt-1	NM_001159920	ATGGTCTTTGCCTGAAATGG	TAGAAGCCAGTGTGGTTTGC
MMP2	NM_001127891	GTGGATGATGCCTTTGCTCG	CCATCGGCGTTCCCATACTT
MMP14	NM_004995	GCAGAAGTTTTACGGCTTGC	ACATTGGCCTTGATCTCAGC
TIMP2	NM_003255	AGAAGAGCCTGAACCACAGG	CTCTGTGACCCAGTCCATCC
FoxM1	NM_001243088	GGGTTTTCTCCTTTGCTTCC	ATGGGTCTCGCTAAGTGTGG

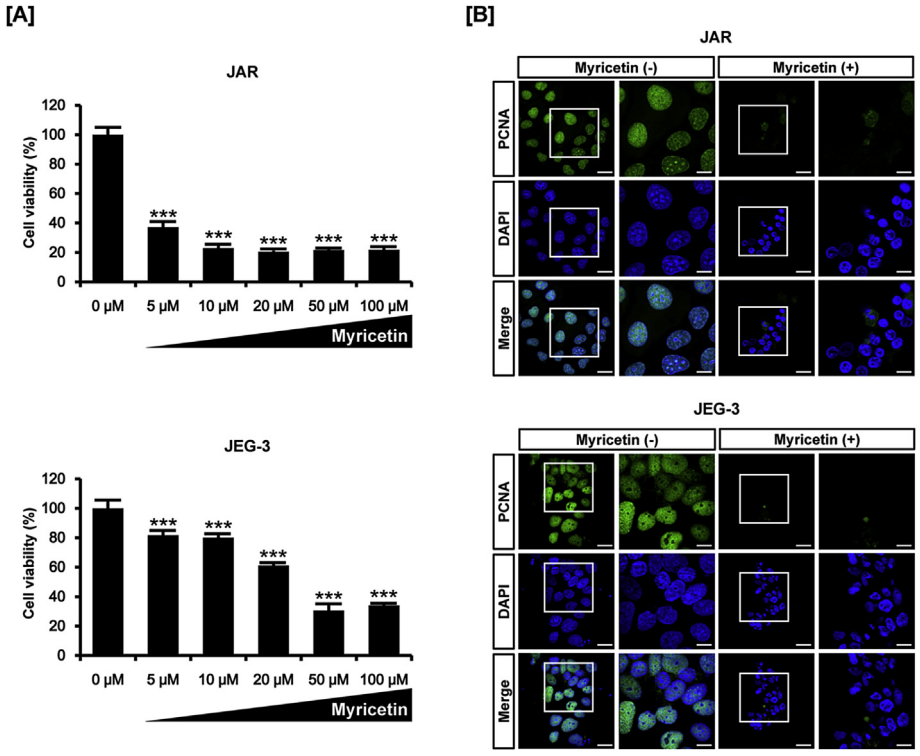

Fig. 1. Effects of myricetin on proliferation of JAR and JEG3 cells. [A] Does-dependent effects of myricetin on the viability of JAR and JEG-3 cells. [B] PCNA protein was detected (green) and nuclei were counterstained with DAPI (blue) in JAR and JEG-3 cells. (***$P < 0.001$). *Scale bar* represents 40 μm (the first and third vertical panels) and 20 μm (the second and fourth vertical panels). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)