Investigation of Apoptotic Effects of Usnic Acid in mRNA Level in Ovarian Cancer SKOV-3 Cell Line

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ABSTRACT

In this study, a lichen secondary metabolite Usnic acid (UA) is applied to ovarian cancer cell line SKOV-3 cells and the apoptotic effect is investigated. The anti-proliferative effect of the UA drug candidate molecule and IC50 concentration on SKOV-3 cells is determined using the XCELLigence Real-Time Cell Analysis (xCELLigence RTCA) assay. In addition, in UA-treated cells, the expression levels of four apoptotic pathway-related genes are determined using real-time quantitative PCR (qRT-PCR). The anti-proliferative impact of UA on SKOV-3 ovarian cancer cells was demonstrated in this study, with a dose- and time-dependent reduction in cell viability after UA treatment.

INTRODUCTION

Ovarian cancer, one of the deadliest types of gynecological cancer in women, is primarily diagnosed in late stages today, and fully effective treatment has not yet been obtained with the conventional treatment methods (1,2). Chemotherapeutics are used as a standard treatment method for ovarian cancer, but with chemotherapeutic treatment methods, promising results are obtained primarily in the early stages of the disease (1,2). The patient develops resistance to chemotherapeutics used in the routine treatment of ovarian cancer stage (2). Additionally, the molecules used in traditional treatments develop toxic effects on the patient over time (2,3). UA is a lichen secondary metabolite abundant in lichenized fungi (4) (Figure 1). UA has some biological effects as anti-cancer, anti-inflammatory and antimicrobial activities (4,5,6).

This study aims to investigate the therapeutic effects of UA as a candidate drug molecule, which is a promising natural and innovative treatment option that has no toxic effect in the treatment of ovarian cancer in addition to existing therapies.

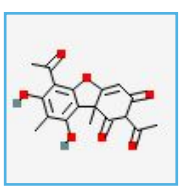

Figure 1. Usnic Acid Molecule [6]

MATERIAL AND METHODS

Cell Culture

Human ovarian cancer SKOV-3 cell line cells were cultured in McCoy's 5A (Modified) Medium (Sigma, USA) with high glucose containing 20% Fetal Bovine Serum (FBS) (Biological Industries, Israel) and 1% penicillin/streptomycin (Biowest, USA) at incubated 37 °C, 5% CO2 (Figure 2).

XCELLigence Real-Time Cell Analysis

In this study, SKOV-3 cells are seeded in XCELLigence Real-Time Cell Analyzer wells, then 1.56, 3.125, 6.25, 12.5, 25, 50 and 100 µM UA and control (DMSO) are added into wells. Cells are inserted into the 5% CO2 media incubator at 37°C for 120 h and their proliferation is monitored with XCELLigence Real-Time Cell Analyzer. RTCA Software Lite software is used to determine the time and IC50 concentration (Figure 3).

RNA Isolation and cDNA Synthesis

SKOV-3 cells were seeded at a density of 5 x 10 cells in 6 well plates, and then the cells were treated with IC50 concentration of UA for 48h. Total RNA Total RNA extraction from UA-treated cells was harvested by Genezol Reagent (Geneaid, Taiwan). The purity and concentration of RNA were determined using ND-1000 Spectrophotometer (Thermo Fischer Scientific, USA) and the integrity was evaluated by performing 1% agarose gel electrophoresis. cDNA synthesis was performed using NG dART Kit (EURx, Poland) by following the manufacturer's instructions (Figure 3).

Real-Time Quantitative PCR (qRT-PCR)

Real-Time Quantitative PCR (qRT-PCR) is performed for four apoptosis pathway-related genes. These genes are *CASP1, CASP3, CASP8* and *BCL-2*. Quantitative RT-PCR reactions were performed using Eva-Green Mix by Roche Light Cycler 480 (Roche) as followed conditions: 10 min at 95 °C, 40 cycles of 10 s at 95 °C, 30 s at 60 °C maintained by final extension for 8 s at 72 °C. GAPDH was used as a reference gene and relative quantification was calculated by the 2−ΔΔCT method (Figure 3).

Statistical Analysis

Expression fold change is calculated according to the 2−ΔΔCT method for qRT-PCR analysis. The student's t-test is performed for statistical analysis.

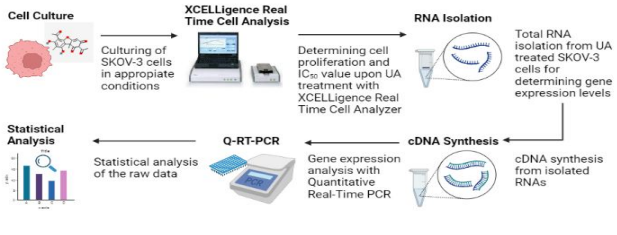

Figure 3. Workflow of the experiments

Figure 2. SKOV-3 cells (40X)

RESULTS

The anti-proliferative effect of UA on SKOV-3 ovarian cancer cells was demonstrated in this study, with a dose- and time-dependent reduction in cell viability after UA treatment. The IC50 concentration of UA is found 8.75 µM, and it is most effective after 10 h of application. The results show that the IC50 concentration of UA (8.75µm) could have significant anti-proliferative effects on the SKOV-3 ovarian cancer cell. Furthermore, qRT-PCR assay revealed that UA application significantly effected gene expression, as evidenced by increases in *CASP 1, CASP3,* and *CASP8* and a significant decrease in *BCL-2* at mRNA level (Figure 4).

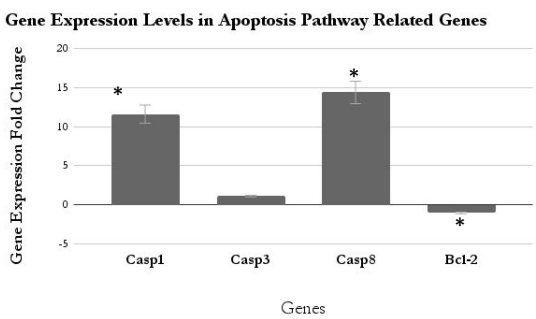

Figure 4. Gene Expression Levels in Apoptosis Pathway Related Genes

DISCUSSION

This study provides results of the anti-proliferative effect of UA treatment on SKOV-3 ovarian cancer cells. The up-regulation of the genes *CASP3, CASP1* and *CASP8* shows that UA induced apoptosis pathway by regulating caspase activity in intrinsic apoptosis pathway. Additionally, UA treatment decreased *BCL-2* levels significantly showing that UA treatment-induced apoptosis also by down-regulating an important anti-apoptotic gene, *BCL-2*. This is the first study in the literature to show that UA has an anti-proliferative and apoptotic effect on SKOV-3 ovarian cancer cells. Therefore UA could be considered as an apoptosis inducer in SKOV-3 cells and might serve as a potential therapeutic candidate in ovarian cancer treatment. Further studies involving in vivo and clinical studies are needed to confirm the therapeutic effect of UA on ovarian cancer.

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