Investigation of Potential Anticarcinogenic Effects of Corilagin in Lung Cancer Cells

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ABSTRACT

Objective: Lung cancer (LC) is the most extensive reason of cancer associated deaths in men and women in the world. LC categorizes into two main groups due to their molecular clinicopathological features and therapeutic responses. Non-small cell lung cancer (NSCLC) is the main subgroup that consists of nearly 85% of all lung cancer types. Corilagin, a biologically active ellagitannin, could be extracted from Phyllanthus species which are known as Chinese medicinal plant. It has been recently shown that Corilagin could exert anti-inflammatory and antioxidative effects in different experimental cancer models. However, the molecular effects of Corilagin in NSCLC remain unclear.

Methods: In this study, the antiproliferative and apoptotic effects of Corilagin were identified by WST-1 cell proliferation test, caspase-3 and mitochondrial membrane potential (MMP).

Results: We found that Corilagin significantly suppressed the proliferation of NSCLC cells. Furthermore, we also showed that Corilagin could contribute apoptosis by inducing activity of caspase-3 molecule and loss of MMP.

Conclusion: Taken together, our study first showed that Corilagin could be a new treatment method for NSCLC after verifying its effects with in vivo and clinical studies.

Keywords: Corilagin; antiapoptotic; non-small cell lung cancer

1. INTRODUCTION

Lung cancer (LC) is the most extensive reason of cancer associated deaths in men and women in the world (1). LC classifies into two subgroups based on their molecular clinical behaviors and therapeutic responses (2). Non-small cell lung cancer (NSCLC) is the main subgroup that consists of nearly 85% of all lung cancer types (3). NSCLC is less responsive to chemotherapeutics in contrast to small cell lung cancer (SCLC) (4). Despite of technological developments on surgical and diagnosis methods, the survival rate of NSCLC patients at metastatic stage is still poor (5-8). Therefore, recent studies turn towards the biomarker and drug researches to find effective and non-toxic treatment methods (9-11).

Corilagin involves in biologically active tannin family which could be extracted from Phyllanthus species (12-15). Previous studies determined that Corilagin could show anti-hypertensive and anti-atherogenic features in experimental models of cardiovascular diseases (16-18). Furthermore, Corilagin could play an important role as a radical scavenger in superoxide anion system. Regarding to its health-beneficial impacts, researchers started to work about whether Corilagin had anticarcinogenic effects for cancer cells with in vivo and in vitro studies (19-23). However, the effects of Corilagin and its molecular mechanisms on LC remain unclear.

To our knowledge, there is no established study about the antitumor effects in NSCLC cells. Herein, our goal was to understand cellular and molecular effects of Corilagin in NSCLC cells as exploring novel and efficient diagnostic methods.

2. METHODS

2.1. Cell Culture

The A549 cell line was kindly picked up from Yusuf Baran (Department of Molecular Biology and Genetics, Izmir Institute of Technology). The cells were maintained in RPMI-1640 enriched with fetal bovine serum and penicillin/streptomycin (37°C, 5% CO2).

2.2. Corilagin Treatment

A549 cancer cells took exposure of dimethyl sulfoxide (DMSO) vehicle (1%) alone or increasing doses of of Corilagin (5, 10, 25, 50 and 100 µM).
2.3. Cell Culture Reagents
The medium, FBS, and penicillin/streptomycin were bought from Gibco Life Technologies (Thermo Fisher; USA). Corilagin was purchased from Sigma Aldrich (Sigma Aldrich; Darmstadt, Germany). The WST-1 was obtained from Roche Life Sciences (Roche Life Sciences; Germany). The Caspase-3 assay was purchased from manufacturer (BioVision; USA). The JC-1 Mitochondrial Membrane Potential Detection assay was obtained from Cayman Chemical (Cayman Chemical; Ann Arbor, MI, USA).

2.4. WST-1 Assay
The cellular impacts of Corilagin in NSCLC cells was determined by using WST-1 assay. The cells were seeded in 96-multiwell plates as a density of $1 \times 10^4$ cells/well. Following incubation period (24h, 48h, 72h), cells were treated with increasing doses of Corilagin (5-100 µM) or vehicle (DMSO, 1%) alone as a control. Ten microliters of WST-1 solution were mixed with cells and then incubated for 4 h at 37°C. The results were read at 450 nm wavelength by using spectrophotometry. Cellular viability was calculated by comparison of proliferation vs. untreated cells (control, 100%).

2.5. Caspase-3 Activity
The colorimetric Caspase-3 assay was to used to determine the apoptotic changes after Corilagin exposure. First, the cells were seeded in 96-well plates (5x10^5 cells/well). After incubation period (24h), cells were induced with Corilagin (50 or 100 µM) or control vehicle (DMSO, 1%) for several time points (24-72h). After each time point, the Corilagin stimulated cells underwent lysis process by adding 50 µL (chilled) and incubated for ten minutes. The supernatants were mixed with 50 µL Reaction Buffer and 5 µL DEVD-Pna substrate then waited two hours for incubation. The results were read at 405 nm wavelength by using spectrophotometry.

2.6. The JC-I assay
The JC-I assay was used to examine the loss of MMP after Corilagin exposure (50 or 100 µM). Briefly, the Corilagin stimulated cells (5 x10^5 cells/2 mL) were collected with centrifugation (1000 rpm, 10 min). After homogenously mixing the remained pellets with 200 µL medium& 20 µL JC-I dye, the cells were incubated for 30 minutes at 37°C and centrifuged. The final pellets were resuspended in 320 µL buffer and then 100 µL of each sample was seeded in a 96-well plate as triplicate. The results were read at Green/red (510 nm/585 nm) wavelengths by using ELISA reader.

2.7. Statistical Analysis
Statistical Package for the Social Sciences software package was used to determine statistical analyzes (revision 11.5 SPSS Inc., Chicago, IL, U.S.A.). The Mann-Whitney U test was used to find the mean value of results. The findings were thought statistically meaningful if the p value was smaller than 0.01 or 0.05.

3. RESULTS

3.1. Corilagin Suppresses A549 Cell Proliferation
We determined that Corilagin stimulation (50 and 100 µM) had statistically significant impacts in A549 cells (*p<0.05,**p<0.01). IC50 values of Corilagin were calculated and found as 0.7 mM (Figure 1).

Figure 1. Effects of Corilagin on cell proliferation. WST-1 proliferation was performed using triplicate samples in three independent experiments. Statistical significance was determined using two-way analysis of variance, and p < 0.05 was considered significant (* p < 0.05; ** p < 0.01).

3.2. Corilagin Improves Caspase-3 Enzyme Activity
We found that there was a 1.5-fold increase in caspase-3 activity after Corilagin treatment (48 h, 50 µM). Furthermore, we also showed that there were 2.6-fold (48 h) and 2.1-fold (72h) changes in caspase-3 activity for several time points after 100 µM Corilagin treatment (Figure 2).

Figure 2. Effects of Corilagin on caspase-3 activity in A549 cells. Changes in caspase-3 enzyme activity in response to increasing concentrations of corilagin in A549 cells. The results are the means of two independent experiments. p < 0.05 was considered significant.
3.3. Corilagin Induces the Loss of Mitochondrial Membrane Potential

The results showed that there was a 1.90-fold changes in the loss of MMP after 50 µM corilagin treatment. In addition, we also found that there were 2.1-fold (48 h) and 1.90-fold (72 h) increases in loss of MMP after 100 µM Corilagin treatment (Figure 3).

**Figure 3. Effects of Corilagin on membrane potential in A549 cells.** Loss of mitochondrial membrane potential (MMP) in response to increasing concentrations of corilagin in A549 cells. The results are represented as the means of two independent experiments. *p < 0.05 was considered significant.*

4. DISCUSSION

In this study, our goal was to examine antiproliferative and apoptotic effects of Corilagin in NSCLC for the first time. Herein, we identified that Corilagin could inhibit cell proliferation of A549 lung cancer cells while promoting apoptotic event like stimulation of caspase-3 activity and loss of MMP. Our finding showed correlation with previous studies. Hau and colleagues verified that Corilagin had antitumoral effects by suppressing tumoral growth in both in vivo and in vitro studies (20). They also showed that Corilagin had no side or toxic effects on liver with using alanine transaminase (ALT) and aspartate transaminase (AST) tests. Furthermore, Gambabi and colleagues found that Corilagin could reverse cellular disruption between intercellular junctions through promoting expression of connexin proteins in lung epithelial cells (24). In the second study, Wang and colleagues examined the impairments of Corilagin in experimental pulmonary fibrosis model. They showed that Corilagin could repair damages in lung epithelial cells by stimulating cytokine secretion and TGF-B activation (25).

5. CONCLUSION

In conclusion, we suggested that Corilagin has potential anticarcinogenic properties to use as a new therapeutic on NSCLC treatment. Further studies will help us to understand the molecular mechanism underlying corilagin stimulation in cancer models.

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**REFERENCES**


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