HOMOISOPOGON A FROM OPHIOPOGON JAPONICUS INDUCES APOPTOSIS IN A549 – A NON SMALL CELL LUNG CANCER CELL LINE

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Received: 23.12.2016
Accepted: 23.3.2017

SUMMARY

Lung cancer is the leading cause of death among Vietnamese people and can be divided into two major groups: small cell lung cancer (SCLC), and non-small cell lung cancer (NSCLC). It is estimated that 85% of patients was diagnosed as NSCLC. Therapy drugs that targeted lung cancer tumors with the epidermal growth factor receptor (EGFR) may initially provide benefit, but over time tumors can develop resistance. The recent discovery of EGFR-tyrosine kinase inhibitors (EGFR-TKIs) has provided a new target for the therapeutic agents in non-small cell lung cancer treatment. In our study, homoisopogon A, a new compound isolated from the tubers of Ophiopogon japonicus (Mach mon, in Vietnamese) effectively showed the cytotoxic activity to the EGFR and TKI-resistant NSCLC cell lines including human lung carcinoma A549, human lung adenocarcinoma NCI-H1975 and human lung adenocarcinoma NCI-H1650. The IC₅₀ values of homoisopogon A against A549, NCI-H1975 and NCI-H1650 were determined as 6.26, 19.51, and 24.66 µM, respectively. Preliminary study on the mechanism of action using flow cytometry analysis was performed. Homoisopogon A treatment of A549 cells at concentration of 25 µM generated apoptosis in 27.5% of cells (7% early apoptosis and 20.5% late apoptosis) after 24h of treatment. The effect increased significantly at the concentration of 50 µM, the homoisoflavanone generated apoptosis in 83.8% of cells (23.5% early apoptosis and 60.3% late apoptosis). Treatment with homoisopogon A for 48h also resulted in typical apoptotic morphological changes in A549 cells under microscopic observation. The results strongly suggested that homoisopogon A induces apoptosis in EGFR and TKI-resistant-A549 cells, thus resulting in the cytotoxicity.

Keywords: Ophiopogon japonicus, homoisopogon A, non-small cell lung cancer (NSCLC), EGFR-TKI, apoptosis

INTRODUCTION

Lung cancer is the most common cause of cancer death in Vietnam and the second deadliest cancers after liver cancer (Bernard et al., 2014). Accordingly, 22,000 Vietnamese people are diagnosed with lung cancer yearly while 19,500 people succumb to the disease every year. Approximately 98% of lung cancers are carcinoma, or tumors derived from transformed cells of epithelial lineage (Gadepalli et al., 2014). Recently, nearly four dozen different histopathological variants of lung carcinoma are recognized (Travis et al., 1995). Lung cancer can be classified into two major types including small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). It is estimated that 85 percent of all lung cancers are non-small cell, and approximately 75 percent of these are metastatic, or advanced, at diagnosis.
Squamous cell carcinoma, adenocarcinoma, and large cell carcinoma are subtypes of non-small cell lung cancer (Travis I 2004). The pivotal role in tumorigenesis of the epidermal growth factor receptor (EGFR) was discovered recently. Accordingly, the EGFR-tyrosine kinase inhibitors (EGFR-TKIs) are considered to be an emerging class of targeted therapeutic agents in NSCLC treatment (Milovancev, 2015). Although most EGFR-mutant NSCLCs initially respond to EGFR-tyrosine kinase inhibitors such as Gefitinib (Iressa) and erlotinib (Tarceva), the vast majority of these tumors ultimately become resistant to the drug treatment (Raz et al., 2006; Bencardino et al., 2011). Currently, EGFR-TKIs are known to contribute considerably to the extension of progression-free survival in prognosis for NSCLC.

In the course to search for anticancer agents, a new homoisoflavanone isolated from the tubers of Ophiopogon japonicus, homoisopogon A, was found to exhibit the potent cytotoxicity against various cancer cell lines including human lung adenocarcinoma LU-1, human epidermoid carcinoma KB and human melanoma SK-Mel-2 cell lines (Dang et al., 2017). This finding suggests homoisopogon A may be a potential therapeutic agent targeted on NSCLC. In this paper, the cytotoxic activity of this compound on different NSCLC cell lines A549, NCI-H1975, and NCI-H1650 was evaluated. The preliminary investigation was conducted to find out whether homoisopogon A is able to induce apoptosis or necrosis in A549 cells using a flow cytometry method.

MATERIALS AND METHODS

Isolation of Homoisopogon A

Homoisopogon A was isolated from the roots of Ophiopogon japonicus as previously described (Dang et al., 2017). Its chemical structure is shown in Figure 1. Homoisopogon A as obtained as amorphous yellow powder, it showed a molecular peak at m/z 345.1 [M + H]+. The purity of homoisopogon A was checked by 1H-NMR and 13C-NMR. The compound was solubilized in dimethyl sulfoxide (DMSO) and used as a final concentration of less than 0.05% DMSO.

Figure 1. Structure of Homoisopogon A.

Cell lines and cell culture

The cancer cell lines A549 (Human lung carcinoma), NCI-H1975 (Human lung adenocarcinoma), NCI-H1650 (Human lung adenocarcinoma) were kindly provided by Prof. Jeong-Hyung Lee, Department of Biochemistry, College of Natural Sciences, Kangwon National University, Korea. The cells were cultured at 37 °C in RPMI 1640 or medium supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin, and 100 µg/ml streptomycin in a 5% CO2 incubator. Cells between 5 and 20 passages were used for the assays.

Cell proliferation assay

An MTT [3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium bromide] assay was used to measure the viability of cells after extract treatment. Cells were seeded in 96-well plates at a density of 2.0×10^4 cells/ml. Cells were treated with various concentrations of the compound (0.3, 1, 3, 10, and 30 µM) and then incubated at 37°C for 48 h. Cells were subsequently incubated at 37°C with MTT (0.5 mg/ml) for 4 h. After removal of supernatant, formazan crystals were dissolved in isopropanol and the optical density was measured at 570 nm (Binh et al., 2015). Camptothecin was used as a positive control.

Morphological changes

A549 cells were treated with different concentrations (1, 10, and 30 µM) of homoisopogon A for 48 hours. The morphological changes of the A549 cells were observed using an inverted light microscope (Olympus CX41, Japan)

Flow cytometry analysis

Apoptosis is assessed by flow cytometry using a FACS equipment (BD Biosciences, USA). The FITC Annexin V Apoptosis Detection Kit I (BD Pharmingen, USA) was used for the assessment. Annexin-V binds to phosphatidylserine (PS) that was externalized early in the process of apoptosis can be detected by the FACS system. A549 cells were plated in 6 cm plates and incubated in 2 mL of medium for 24 h. After the incubation, cells were
treated with homoisopogon A at two concentrations 25 µM and 50 µM and incubated for 24 h. Cells were then collected, washed once with PBS, and resuspended in the 1X Binding buffer at a density of 1 × 10⁶ cells/ml. FITC Annexin V and PI was added and incubated for 15 min, at room temperature, in darkness. The cells were analyzed in the FACS equipment within 1 hour.

Statistics

Results are given as the mean standard error for the mean (SEM). IC₅₀ values and statistical analyses were performed with GraphPad Prism 6.0 Software (La Jolla, CA, USA).

RESULTS AND DISCUSSION

In the past few years, the discovery of the pivotal role in tumorigenesis of the epidermal growth factor receptor (EGFR) provided a new class of targeted therapeutic agents: the EGFR-tyrosine kinase inhibitors (EGFR-TKIs) (Milovancev et al., 2015). Interestingly, homoisopogon A exhibited strong cytotoxic effect to the wild type of EGFR-TKI-resistant A549 cells (IC₅₀ = 6.26 ± 0.79 µM), which was more effective than to the positive control, camptothecin (IC₅₀ = 12.42 ± 0.56 µM). Also, homoisopogon A exhibited the strong cytotoxicity toward two other cell lines NCI-H1975 and NCI-H1650. The cytotoxicity of homoisoflavonoids has been reported elsewhere (Lin et al., 2014; Alali et al., 2015), however, this is the first report on the effects of the homoisoflavanone on different EGFR/TKI-resistant lung cancer cell lines.

Remarkably, homoisopogon A seems to be more specific on the wild type of EGFR and TKI-resistance than the mutation ones. This finding may provide a new agent targeting on the wild type of EGFR/TKI-resistant cell lines. We continued to further investigate the mechanism of action of this compound in A549 cells.

Figure 2. Effects of homoisopogon A at different concentration (A: 0 µM; B: 1 µM, C: 10 µM; D: 30 µM) on the morphological changes of A549 cells after 48h treatment.
Table 1. Cytotoxic effect of homoisopogon A towards NSCLC cell lines (IC\textsubscript{50} ± SD, µM).

<table>
<thead>
<tr>
<th>Cell lines</th>
<th>Wild type of EGFR and TKI-resistance</th>
<th>Mutation of EGFR and TKI-resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homoisorophon A</td>
<td>6.26 ± 0.79</td>
<td>24.66 ± 0.09</td>
</tr>
<tr>
<td>Camptothecin\textsuperscript{a}</td>
<td>12.42 ± 0.56</td>
<td>8.23 ± 0.56</td>
</tr>
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</table>

\textsuperscript{a} positive control. All experiments were carried out in triplicated.

Figure 3. Apoptotic effect of homoisopogon A in A549 cells analyzed with Annexin V-FITC/PI assay after 24h treatment.

Cell death by apoptosis usually result in the morphological changes. The most significant characteristic changes are cell shrinkage, nuclear condensation and membrane blebbing with the formation of apoptotic bodies. Figure 2 shows the growth of A549 cells was inhibited by the treatment with homoisopogon A for 48 hours. The number of cells decreased and cell debris increased in the proportion with the increase of drug concentration. Cells exhibited the typical apoptotic morphological changes including both cell shrinkage, nuclear condensation and membrane blebbing (Fig. 2C, D).

Apoptosis and necrosis are the two major types of cell death with different morphological characteristics (Farber, 1994). We next investigated whether necrosis or apoptosis is involved in the mechanism of action of homoisoflavone A in the EGFR-TKI-resistant A549 cells. FACS analysis using annexin V-FITC staining and PI accumulation was employed to differentiate early apoptotic cells (annexin V positive and PI negative) from necrotic or late apoptotic cells (annexin V positive and PI positive). Interestingly, homoisopogon A induced apoptosis potently (Fig. 3A) at two investigated concentrations after 24h of treatment. Homoisopogon A treatment of A549 cells at concentration of 25 µM generated apoptosis in 27.5% of cells (7% early apoptosis and 20.5% late apoptosis). The effect increased significantly at the concentration of 50 µM, the homoisoflavanone.
generated apoptosis in 83.8% of cells (23.5% early apoptosis and 60.3% late apoptosis) (Fig. 3B). The movement of cells strongly suggested the cells underwent the apoptosis by treatment of homoisopogon A.

The growth factor EGF is one of the major promoters of migration and mobility in cancer cells. It regulates the cell proliferation, growth and differentiation by binding to its receptor EGFR. Two important cascades that can be activated by EGF are PI3K/Akt and MAPK/ERK pathways. Inhibition of the PI3/Akt pathway induces cell apoptosis in most cancers (Mao et al., 2016). Whereas, the MAPK/ERK pathway plays a role in linking the extracellular signals to control the cellular process such as cell migration, proliferation, and apoptosis (Dhillon et al., 2007). Inhibition of EGFR, therefore, may suppress the EGF activation and result in the apoptosis of the cancer cells. Our preliminary findings indicated that the homoisoflavonane might have potential as an anti-cancer agent, and further studies are being performed to clarify its mode of action.

CONCLUSIONS

Our study indicated that homoisopogon A isolated from Ophiopogon japonicus showed the cytotoxicity to three lung cancer cell lines including A549, NCI-H1975 and NCI-H1650. Preliminary study on the mechanism of action revealed that homoisopogon A induces apoptosis in A549 cells at two different concentrations after 24h of treatment.

Acknowledgements: This work is supported by the National Foundation for Science and Technological Development (NAFOSTED 104.01-2014.05).

REFERENCES


