Decursin was Accelerated Human Lung Cancer Cell Death Caused by Proton Beam Irradiation via Blocking the p42/44 MAPK pathway.

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1. Introduction

Decursin, which is one of the extract of *Angelica gigas* Nakai root, has been traditionally used in Korean folk medicine as a tonic and for treatment of anemia and other common diseases. There are some reports about the pharmacological properties of decursin showing anti-bacterial and anti-amnestic effect, depression of cardiac contraction, antitumor and anti-angiogenic activity [1-3].

Cell death induced by proton beam is identified as apoptosis [4]. The study investigated that genes involved in apoptosis are checked by RT-PCR and used LET instead of SPBP of proton beam. Apoptosis is the tight regulated by multi-protein action in physiological cell death program.

Proton therapy is an attractive approach for the treatment of deep-seated tumor. Recently, many researchers tried to new therapeutic strategy, combination of proton therapy and chemotherapy, in order to increase therapeutic effect. In this study, we investigate whether decursin can accelerate effect of human lung cell apoptosis in proton irradiated cancer cells.

2. Methods and Results

2.1 Chemicals and Antibodies

Rabbit polyclonal antibodies against ERK, p-ERK, JNK, p-JNK, p38, p-p38, bad, bax, caspase-9, c-caspase-9, PARP, and p-PARP were purchased from Cell Signaling and mouse monoclonal antibodies against beta-actin, STAT-1, Bcl-xL and CDK-2 were purchased from Santa Cruz. Other chemicals were obtained from Sigma Aldrich.

2.2 Plant Extracts and Purification

The roots of *Angelica gigas* Nakai was extracted serially with methanol, ethylacetate, and n-buthanol, and fractionated. From the ethylacetate faction, Decursin was isolated using silica gel column chromatography. After column chromatography, the structure of purified coumarin compound decursin with a molecular weight of 328 were characterized by gas chromatography, nuclear magnetic resonance and mass spectroscopy. The structure and NMR spectra of Decursin were shown in Fig. 1, respectively.

2.3 Cell culture

Human lung cancer cells (A549) were grown on culture plate in DMEM supplemented with 10% fetal bovine serum, and 1x antibiotics.

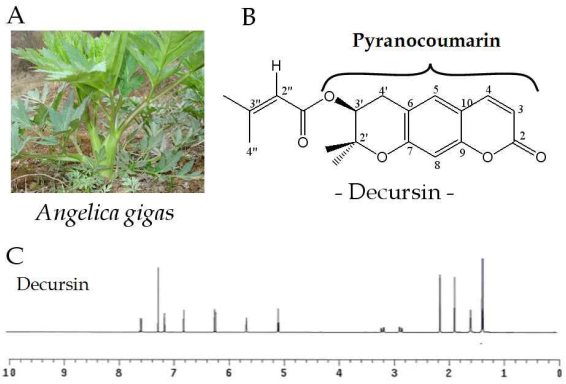

Figure 1. Structure and NMR spectra of Decursin. A. The picture of *Angelica gigas* Nakai, B. Chemical structure of Decursin, C. Purified Decursin was characterized by NMR and mass spectroscopy.

2.4 Proton beam irradiation.

On one day after seeding, cell irradiated with proton beam at mean current of 10 nA and 0.5Gy/sec dose rate. We used MC-50 cyclotron at the Korea Istitute of Radiological and Mecical Sciences (KIRAMS). A T-25 or T-12.5 flask or 96-well plate was used to fit beam geometry, on the cells were plated. For the same LET or dose rate condition, we used SOBP system.

2.5 Cytotoxic test

A549 cells seeded in 96-well plates $2x10^3$ cells / well. After 24h, Decursin was added and incubated for 16h, and then, proton beam irradiation. Cytotoxicity was determined using MTT assay. Figure 2 shows that cancer cell death is occurred by proton beam irradiation. And added decursin significantly inhibited cell survival by 10 to 20 μ M in a dose-dependent manner. Survival rate is also decreased by proton beam and decursin by time-dependent manner.

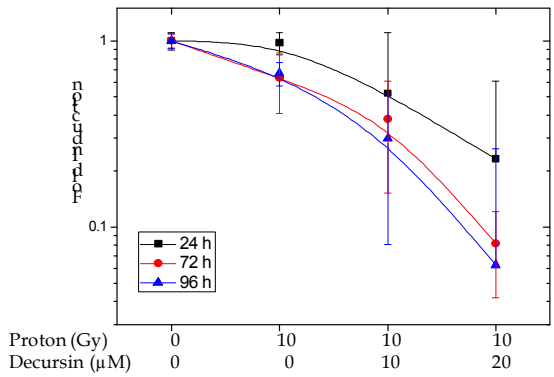

Figure 2. Cytotoxicity test of decursin and proton beam irradiation.

2.5 Western blot analysist

Western blotting analysis was performed as previously described [5]. Because decursin and proton beam induce tumor cell death. We determined whether they can inhibit apoptosis signaling cascade pathway. First, we examined MAPK activation (figure 3). Decursin and proton beam treated groups significantly suppressed ERK phosphorylation compared with only proton beam induced down-regulated ERK phosphorylation. However, phosphor-Akt were activated by the treatment of decursin, Indicating activation of MAPKs and inactivation of survival kinases are both involved in proton –induced apoptosis.

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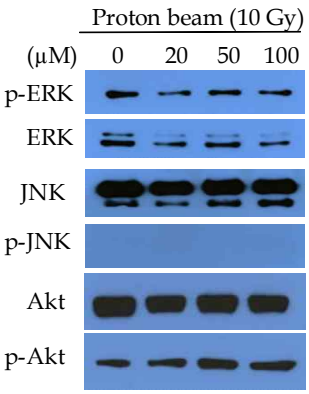

Figure 3. Proton beam and decursin suppress ERK signaling pathway in A549 cells

Next, to investigte the mechanisms by which proton beam and decursin treated cancer cells induce apoptotic cell death, we performed western blot analysis with antibodies against apoptosis-related proteins. Procaspase-9 was decreased by decursin treated cells. STAT-1 and bad protein also decreased by decursin dose-dependent manner

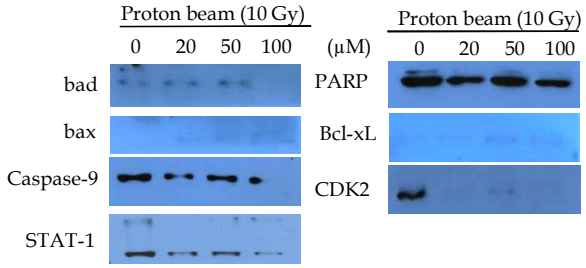

Figure 4. Proton beam and decursin inhibit CDK2-STAT-1 pathway.

3. Conclusions

Decursin and proton beam co-treated groups significantly induced tumor cell apoptosis compared with proton beam treated groups. Decursin blocked STAT1 activation in a dose-dependent manner in A549 cells. These results suggest that decursin may be a potential candidate for sensitizer of proton beam induced cell apoptosis

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