Decursin reduce radio-resistance of hypoxic regions under the proton beam therapy by induced HIF-1α degradation

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1. Introduction

The proton beam is well-known as a one of the attractive methods for the treatment of tumor tissues. The proton has a better dose distribution compared to conventional photons, such as X-ray or gamma-ray [1, 2]. Due to the physical advantage of proton, it can be reduce radiotherapy-induced side effects of sparing normal tissue. Proton beam-induced cell death is identified as apoptosis by ROS (Reactive Oxygen Species) via the making by water hydrolysis [3]. Mechanisms of proton beam-induced cell death in in-vitro are partly clear. Also, protons induce cancer-cell apoptosis in vitro and block blood vessel formation in vivo through the generation of reactive oxygen species (ROS) [3, 4]. The fact that proton severely inhibits blood vessel development in zebrafish embryos [4] suggests a higher sensitivity of vascular endothelial cells to proton beam.

Decursin, a coumarin compound, was originally isolated from Angelica gigas Nakai (Dang Gui). A. gigas root has been traditionally used in Korean folk medicine for the treatment of anemia and other common diseases. In previous reports, decursin was reported to exhibit anti-tumor activity against various cancer cells [5-7] and to inhibit the activities of the androgen and androgen-receptor (AR) signaling pathway in prostate cancer [8], induction of cell cycle arrest and apoptosis in various cancer cells, such as prostate, breast, bladder, and colon cancer cells [9-11]. Decursin also inhibits VEGF-induced angiogenesis through the suppression of the VEGFR-2-signaling pathway [12]. However, the mechanism of decursin mediates change of HIF-1αactivities is not clear.

In this research, we identified regulations of the HIF-1αand the anti-angiogenesis effects of decursin in proton-beam-irradiated human lung cancer, prostate cancer and Hepatic cancer cells. We investigated the underlying mechanisms of positive effects of proton-beam-induced anti-angiogenesis. Our data indicate that the groups co-treated with decursin and a proton-beam had significant reduced HIF-1α activity compared with the groups treated with only a proton beam under the hypoxic condition caused by DFX(desferrioxamine). Decursin was found to induced HIF-1αdegradation. Therefore, we suggest that decursin may be a potential candidate for use as a sensitizer for proton-beam-induced cell apoptosis

2. Methods and Results

2.1 Cell culture

Hep3B human hepatocellular carcinoma cells, A549 human lung cancer cells HCT116 human prostate cells were maintained in DMEM or RPMI supplemented with 10% FBS (heat inactivated, Hyclone, Logan, UT) and 1 × antibiotics [100 U/ml penicillin, 100 μg/ml streptomycin, all from Invitrogen (Carlsbad, CA)] at 37°C in a humidified atmosphere incubator containing 5% CO2.

2.2 Chemicals and antibodies

YC-1(HIF-1α inhibitor) was purchased from Sigma Aldrich. Mouse monoclonal antibodies against HIF-1α, VEGF-A and β-actin were purchased from Santa Cruz Biotechnology (Santa Cruz, CA) and Zymed (San Francisco, CA), respectively.

Decursin (Fig. 1) was purchased from Natural Product Bank at the Institute for Korea Traditional Medicine Industry.

Pyranocoumarin

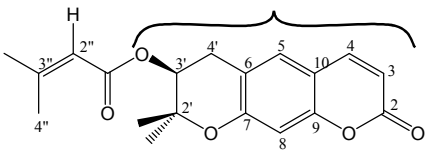

Fig. 1. Photograph of Angelica gigas (Top) and structure of decursin (Bottom)

2.3 Proton beam irradiation and dosimtery

On a day after plating, cells were irradiated with proton beam. At the 100-MeV proton linac at the Korea Multi-purpose Accelerator Center, Gyeong-ju, Korea, 100 MeV protons were produced at the Bragg peak. A 25 cm2 flask or 96-well plate was used to fit beam

geometry, on which the cells were plated. The beam was extracted for SOBP with which the high LET was repeated at broad region as a function of depth of flask or plate and media on which the cells were plated. It was assumed that the thickness of the cell monolayer was between 3-6 μm and that of the media was 2 cm. Just before the irradiation, the flask or 96-well plate was fixed vertically with a tape, facing the horizontal beam. Cells were irradiated at the chosen position at single dose level of 5 Gy.

2.4 Decursin induced HIF-1αdegradation and reduce radio-resistance under hypoxic condition.

Aiming to clear the mechanism for the anti-angiogenic activity of decursin, we examined whether decursin affects HIF-1a stabilization, which occur under hypoxia mimic condition. A549, Hep3B and HCT116 cancer cell were pre-exposed to 8 hrs and hypoxia generated chemically with desferrioxamine for the last 12 hrs and then proton beam irradiation by 5 Gy dose. The results show that exposure to 20 μM of decursin significantly increased cell death and reduced HIF-1α stability

3. Conclusions

Here we have shown that decursin successfully reduced HIF-1α stability under hypoxic condition by induced desferrioxamine. We showed novel candidates for anti-angiogenic compound, decursin, leading to complete inhibition of radio-resistance of proton beam in the A549, Hep3B and HCT116 cells under hypoxic condition via degradation of HIF-1α.

In summary, our results provide a novel function of decursin as anti-angiogenesis agent by targeting HIF-1α stability. Therefore, we suggest that decursin may be a potential candidate for use as a sensitizer for reduced radio-resistance of proton-beam.

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