AMRI-59 has a role of radiosensitizer via enhancement of γ-ionizing radiation-induced apoptotic cell death.

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1. Introduction

As non small-cell lung cancer (NSCLC) has an extremely low 5-year survival rate [1], many researchers have concentrated to increase patients' recovery. Radiotherapy using ionizing radiation (IR), chemotherapy and surgery are extensively used for the treatment of NSCLC [2]. However, the clinical usefulness of IR may be limited by problems with induction of radioresistance and damage to normal adjacent tissues [3]. Moreover, recent *in vitro* studies have suggested that may increase the invasiveness of some cancer cells (e.g., glioma, hepatocellular carcinoma, and pancreatic cancer cells) by stimulating several intracellular signaling pathways and *in vivo* studies have found that radiotherapy of primary tumor sites may promote metastasis [4–6]. Thus, in addition to having therapeutic effects, IR might promote the malignant traits of surviving cancer cells. The existing efforts to develop radiosensitizing agents have focused on overcoming radioresistance and reducing damage to normal tissues. Recently, concepts of personalized- or precision medicine are developed due to advancement of mega data technique, which provide new targets to develop new anti-cancer drugs. In this study, we sought to identify the radiosensitizer effect of AMRI-59 *in vitro* and *in vivo*., which is recently developed specific inhibitor of peroxiredoxin (Prx) I [7].

2. Methods and Results

2.1 AMRI-59 has a role of radio-sensitizer effect against NSCLC in vitro.

NCI-H460 cells were purchased from ATCC (American Type Culture Collection, Manassas, VA) and cultured in RPMI1640 (Invitrogen Co., Grand Island, NY) containing 10% heat-inactivated fetal bovine serum (Invitrogen Co., Grand Island, NY), sodium bicarbonate (2 mg/ml, Invitrogen Co., Grand Island, NY), penicillin (100 units/ml) and Streptomycin (100 μg/ml, Invitrogen Co., Grand Island, NY) and maintained at 37℃ in a 5 % CO_2 incubator. AMRI-59 (N- (3-acetylphenyl)-4-(biphenyl-4-ylmethyl) piperidine-1-carboxamide) came from AMRI (Albany, NY, USA).

To detect radiosensitizer effect of AMRI-59, we performed clonogenic assay as follows; NCI-H460 cells were seeded in triplicate 60-mm dishes at cell concentrations estimated to yield 20-100 colonies/dish. After 24 hours of incubation, cells were treated with 5, 10, 20 5 μM of AMRI-59 for 24 hours and then irradiated using a ^{137}Cs as a source of γ-ray (Atomic Energy of Canada, Ltd., Ontario, Canada) at various doses (2, 5, or 10 Gy). Cells were cultured for 14 days and colonies larger than 200 μm in diameter were counted using a colony counter (Imaging Products, Chantilly, VA) in Figure 1. We also detected cell death using PI uptake (Fig. 2). Cells were seeded at a density of 1×10^5 cells and incubated with or without 20 μM AMRI-59. After 16 hours of incubation, cells were exposed to 3 Gy IR, and then incubated for 48 hours. Treated cells were harvested by trypsinization, washed twice with cold PBS, and resuspended in 300 μL of a 5-μg/mL propidium iodide (PI, Sigma-Aldrich) solution. The apoptotic fraction was evaluated using a FACSort flow cytometer (Becton Dickinson).

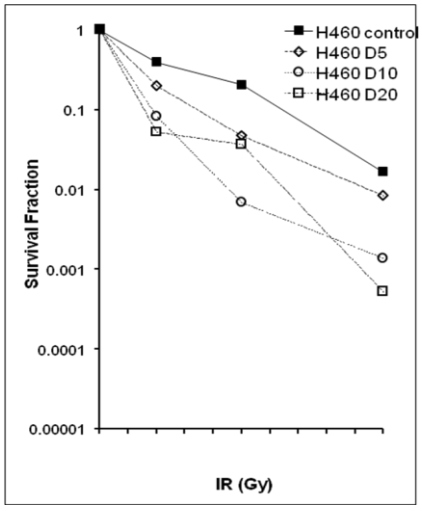

Fig. 1. Clonogenic assay for detection of radiosensitizer effect of AMRI-59 against NCI-H460 .

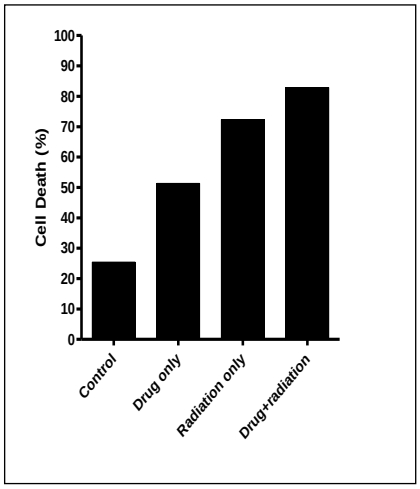

Fig. 2. Combined treatment of IR and AMRI-59 induced apoptotic cell death.

2.2. AMRI-59 shows a role of radio-sensitizer effect against NSCLC in vivo.

As AMRI-59 has a role of radiosensitizer effect against NSCLC *in vitro*, we tested whether AMRI-59 also enhanced cell death with IR treatment *in vivo*. NCI-H460 cells (1×10^7) were subcutaneously injected into 6-week-old BALB/cAnNCrj-nu/nu strain mice (Charles River Japan Inc., Japan) to evaluate the *in vivo* effect of PHCM. When these xenografts reached 120 mm^3, 50 or 100 mg/kg of AMRI-59 was subcutaneously injected into the tumor sites. IR-only treated or control groups were injected with equal volumes of the vehicle solution (DMSO), and IR-only treated or combination groups were irradiated with 5 Gy after 7 hours. This experiment was repeated 4 times at 5-day intervals and tumor sizes were detected over 55 days. The volume of each xenograft was calculated (short $axis^2$ × long axis/2) to determine tumor volumes. Mice were anesthetized intraperitoneally (100 μl) with a narcotic drug combination [Xylazine HCl (Bayer Korea Co., Seoul): Ketamine HCL (Yoohan Co., Seoul): PBS (phosphate-buffered saline) = 0.15 (v): 0.85 (v): 1 (v)], and then fixed on an acryl plate and locally irradiated with a ^{60}Co γ-ray source (Theratrom 780, AECL Ltd., Mississauga, Ontario). The body parts other than tumor xenografts were protected with lead blocks.

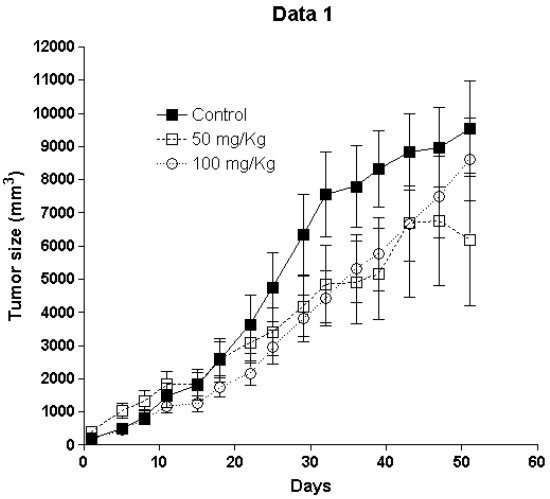

Fig. 3. Combination of AMRI-59 and IR promotes regression of tumor mass *in vivo*.

3. Conclusions

AMRI-59 enhanced radiation-induced cell death and its mean calculated dose enhancement ratio was 1.26. We also found combination of AMRI-59 and IR In a xenograft assay, the combined PHCM and radiation group showed 14.3 days of growth delay versus the control in terms of tumor growth. The enhancement factor of this combined treatment was determined to be 2.03.

REFERENCES

[1] Siegel R, Ma J, Zou Z, Jemal A. Cancer Statistics, 2014. CA Cancer J Clin 2014;64:9–29.

[2] B. Martini, Lung cancer–epidemiology, prognosis and therapy. Med Monatsschr Pharm 2006;29: 217–21.

[3] Begg AC, Stewart FA, Vens C. Strategies to improve radiotherapy with targeted drugs. Nat Rev Cancer 2011;11: 239–53.

[4] Park CM, Park MJ, Kwak HJ, Lee HC, Kim MS, Lee SH et al. Ionizing radiation enhances matrix metalloproteinase-2 secretion and invasion of glioma cells through Src/epidermal growth factor receptor-mediated p38/Akt and phosphatidylinositol 3-kinase/Akt signaling pathways. Cancer Res 2006;66: 8511–9.

[5] Cheng JC, Chou CH, Kuo ML, Hsieh CY. Radiation-enhanced hepatocellular carcinoma cell invasion with MMP-9 expression through PI3K/Akt/NF-kappaB signal transduction pathway. Oncogene 2006;25:7009–18.

[6] Ho JN, Kang GY, Lee SS, Kim J, Bae IH, Hwang SG et al. Bcl-XL and STAT3 mediate malignant actions of gamma-irradiation in lung cancer cells. Cancer Sci 2010;101: 1417–23.

[7] Yang YJ, Baek JY, Goo J, Shin Y, Park JK, Jang JY, Wang SB, Jeong W, Lee HJ, Um HD, Lee SK, Choi Y, Rhee SG, Chang TS. Effective killing of cancer cells through ROS-mediated mechanisms by AMRI-59 targeting peroxiredoxin I. Antioxid Redox Signal. 2015 Nov 3. [Epub ahead of print]