RIP1 controls IR-induced invasion of non-small cell lung cancer cells

Jeong Hyun Cho[a], Ju Yeon Kim[a], EunAh Lee[b], Jie-Young Song[a], Sang-Gu Hwang[a], Hong-Duck Um[a], and Jong Kuk Park [a†]

[a]Division of Applied Radiation Bioscience, Korea Institute of Radiological and Medical Sciences, Seoul 01812, Republic of Korea

[b]Impedance Imaging Research Center, Kyung Hee University, Seoul, Korea

[*]*Corresponding author*

1. Introduction

γ- Ionizing radiation (IR) is extensively used in cancer therapy (1–3). But, some of previous studies showed that IR increases the invasiveness of cancer cells including glioma, hepatocellular carcinoma, pancreatic cancer cells. Moreover, some research result presented that local radiotherapy administered to primary tumors speeds their metastatic growth *in vivo* (4-6), thereby suggesting that besides its therapeutic effects, IR promotes the malignant behaviors of surviving cancer cells. RIP1 have critical role with cell stress create by many factors, such as inflammation and DNA damage. (7) RIP1 is an important regulator of cell survival and is a pivotal component of the inflammatory-signaling pathway. (8) In this study, we identify the mechanism of RIP1 in IR activated EMT.

2. Methods and Results

2.1 IR induced of invasion and migration and expression RIP1.

Human A549 lung cancer cells were cultured in RPMI-1640 medium supplemented with 10% heat-inactivated fetal bovine serum. Cells were maintained at 37℃ in 5% CO_2. For all experiments using IR-treated cells, A549 cells (5×10^5) were seeded to 60-mm dishes and incubated overnight. IR exposure (10 Gy) was performed using ^{137}Cs as a radiation source (Atomic Energy of Canada, Ltd., Mississauga, ON, Canada). The IR-treated cells were used for experiments after 24 h (Fig. 1A).

Matrigel coated transwells (Corning NY, USA) were performed cell invasion and Collagen coated transwells (Corning NY, USA) were performed cell migration. Cells ($1x10^4$) in 200 ul of medium were seeded onto the upper chamber in transwells. Serum-free media supplemented with 0.1% bovine serum albumin in lower chamber. Transwells were incubated for 18h with 5% CO_2 at 37 ℃. Cell staining was performed with deep quick solution (Merck, Whitehouse Station, NJ,USA) (Fig. 1A).

RIPA buffer [50 mM Tris, pH.8.0, 150 mM NaCl, 1% NP-40, 0.5% deoxycholic acid, and 0.1% sodium dodecyl sulfate (SDS)] containing protease and phosphatase inhibitor was used to dissolve harvested cell pellets for acquiring whole-cell protein lysates. Cell lysates were separated by 12% SDS -polyacrylamide gel electrophoresis (PAGE) and protein were transferred to nitrocellulose membranes. The membrane was incubated with blocking buffer (containing 5% skim milk in 0.1% PBS-T) at room temperature and then was washed in 0.1% PBS-T. After theses step, the blot was incubated with protein-specific primary antibody and then was incubated with labeled with HRP conjugated secondary antibody. Finally, the blot was treated with ECL and was exposed film in the darkroom (Fig. 1B)

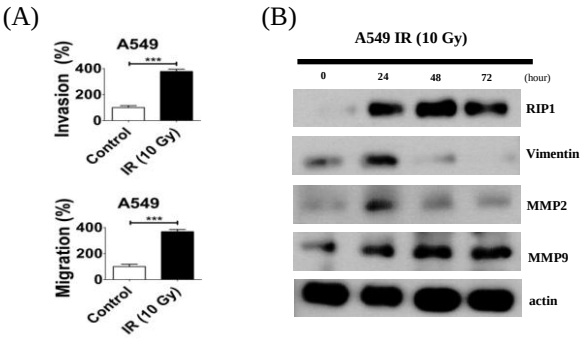

Fig. 1. (A) IR increased invasion and migration. (B) IR induced RIP1 and EMT pathway in A549 cell.

2.2. Ncerostatin-1 treatment regulated EMT pathway

As previous data indicates IR could induce RIP1 and EMT pathway simultaneously, we test whether RIP1 could affect IR-induced invasion and migration. We pre-treated necrostatin-1, specific pharmacological inhibitor of RIP1, to A549 cells after IR irradiation and performed migration & invasion assay as well as

detection of EMT pathway with performing of immunoblotting assay. (Fig. 2)

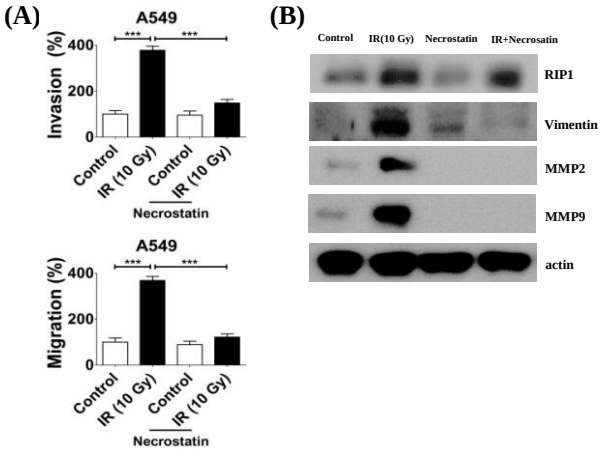

Fig. 2. Necrostatin-1 decreased IR-induced invasion/migration and EMT pathway.

3. Conclusions

Previous data has shown that IR enhances the invasiveness of A549 cells (8). IR stimulates a cellular pathway involving Src, STAT3 and MMP-2/9 in A549 cells. These effects of IR were also accompanied by EMT-associated events including an increase in RIP1 levels. Inhibition of RIP-1 by necrostatin-1 suppressed enhancement of IR-induced invasiveness effect and activation of EMT pathway in A549 cells. These results suggest that RIP1 is involved in EMT pathway and may be used as a target of improve the efficacy of radiotherapy.

REFERENCES

[1] Schmidt-Ullrich RK, Molecular targets in radiation oncology, Oncogene 2003;22: 5730–3.
[2] Barcellos-Hoff MH, Park C, Wright EG, Radiation and the microenvironment tumorigenesis and therapy, Nat Rev Cancer 2005; 5: 867–75.
[3] Kumar P, Miller AI, Polverini PJ, Ap38 MAPK mediates γ-irradiation induced endothelial cell apoptosis, and vascular endothelial growth factor protects endothelial cells through the phosphoinositide 3-kinase-Akt-Bcl-2pathway, J Biol Chem 2004; 279: 43352–60.
[4] Canphausen K, Moses MA, Beecken WD, Khan MK, Folkman J, O'Reilly MS, Radiation therapy to a primary tumor accelerates metastatic growth in mice, Cancer Res 2001; 61: 2207–11.
[5] Biswas S, Guix M, Rinehart C et al, Inhibition of TGF-beta with neutralizing antibodies prevents radiation-induced acceleration of metastatic cancer progression, J Clin Invest 2007; 117: 1305–13.
[6] Madani I, De Neve W, Mareel M, Does ionizing radiation stimulate cancer invasion and metastasis? Bull Cancer 2008; 95: 292–300.
[7] Duanwu Zhang, Juan Lin and Jiahuai Han, Receptor-interacting protein (RIP) kinase family, Cellular & Molecular Immunology (2010) 7, 243–249
[8] F Humphries, S Yang1, B Wang and PN Moynagh, RIP kinases: key decision makers in cell death and innate immunity, Cell Death and Differentiation (2015) 22, 225–236
[9] Jong Kuk Park, Seon Ho Park, Kwang Sup So, In Hwa Bae, Young Do Yoo and Hong-Duck Um, ICAM-3 enhances the migratory and invasive potential of human non-small cell lung cancer cells by inducing MMP-2 and MMP-9 via Akt and CREB, International Journal of Oncology 36: 181-192, 2010.
[10] Jin-Nyoung Ho, Ga Young Kang, Seung-Sook Lee, Jongdoo Kim,1 In Hwa Bae, Sang-Gu Hwang and Hong-Duck Um, Bcl-XL and STAT3 mediate malignant actions of γ-irradiation in lung cancer cells, Cancer Sci | June 2010 | vol. 101 | no. 6 | 1417–1423.