http://dx.doi.org/10.5392/IJoC.2012.8.4.073

The Regulatory Effects of Radiation and Histone Deacetylase Inhibitor on Liver Cancer Cell Cycle

Sang Ho Lee[1], Chang Hee Han[1], Su Man Kang[2], Cheol Woo Park[3]
Department of biology Dong-eui University, Busan, 614-714, South Korea[1]. Department of Radiation Onoldgy, Kosin University Gospd Hospital[2], Department of Radiologic Technology Dong-eui Institute of Technology, Busan, 614-715, South Korea[3]

ABSTRACT

Radiation has been an effective tool for treating cancer for a long time. Radiation therapy induces DNA damage within cancer cells and destroys their ability to reproduce. Radiation therapy is often combined with other treatments, like surgery and chemotherapy. Here, we describe the effects of radiation and histone deacetylase inhibitor, Trichostain A, on cell cycle regulation in hepatoma cells. The combinatorial treatment of radiation and Trichostain A induced cell cycle arrest and thereby increasing the hepatoma cell death. Furthermore, the regulatory effects of radiation and Trichostatin A on cell cycle applied in cell type specifically. These results suggest that the treatment of radiation and Trichostatin A may play a central role in hepatoma cell death and might be a good remedy to improve the efficiency of radiation therapy.

Keywords: *radiation, Trichostatin A and liver cancer*

1. INTRODUCTION

In eukaryotic cells, genomic DNA is well organized into a nucleoprotein structure called the nucleosome, consisting of repeating octamers of four core histones wrapped around 146 bp of DNA. [1]-[3]. The histone N-terminal tails are directly involved in transcriptional regulation by their reversible modifications, including methylation, acetylation, pho sphorylation, and ubiquitination [6], [11]. Among diverse chromatin remodeling processes, acetylation of histone N-terminal tails has been implicated in transcriptional activation [4], [5], [7], [9]. When cells are exposed to specific conditions, activators recruit histone acetyltransferases (HATs) and establish distinct histone acetylation on its target sites. Increased acetylation levels permit the transcriptional machinery to access the promoters and initiate the high level of transcription [4], [5], [7], [9]. Histone acetylation is a reversible process, which is dynamically governed by the opposing activities of HAT and histone deacetylase (HDAC). For the initiation of activator-mediated transactivation, HAT activity overcomes HDAC activity to establish promoter-targeted histone acetylation, thereby resulting in active transcription. Although acetylation of all four core histones has been linked to transcriptional activation, recent studies indicate that acetylation of H3 and H4 plays a critical role in radiation-inducible target gene expression [4], [5], [7], [9].

Ionizing radiation is a well known genotoxic agent and a human carcinogen [12]. Radiation-induced genomic instability manifests as reduced clonogenic survival, chromosomal aberrations, and/or gene mutations [8]. However, radiation also influences the regulation of tumor growth and death through the cell cycle regulation. Along with radiation, histone deacetylase inhibitor, Trichostatin A (TSA), also regulates the cell cycle in various cancers, thereby stimulating tumor cell death [10]. Although radiation and TSA have been widely studied in cancer, the regulatory mechanism of cell cycle by radiation and TSA in liver cancers remains unknown.

Here, we show that altered cell cycle regulation mediated by radiation and TSA stimulates the death of two different hepatoma cell lines, HepG2 and Hep3B. Moreover, we observed that these two cell lines have different sensitivity for radiation and TSA leading to the different cell cycle regulation.

2. MATERILAS AND METHODS

2.1 Microsopy analysis

2×10^5 cells were seeded in 6-well plates, and incubated at 37°C for 24hr. Cells were treated with TSA and incubated for 24hr at 37°C, followed by radiation. After 24 hr, cells were analyzed using a x 40 NA 0.75 objective on an inverted microscope (Nikon Instruments Inc.).

2.1 Cell proliferation assay (MTT assay)

Cell proliferation was determined using the MTT assay.

* *Corresponding author: riroom@hanmail.net*
Manuscript received Sep. 21, 2012; revised Nov 25, 2012; accepted Dec 05, 2012

2×10^5 cells were seeded in 6-well plates, and incubated at 37°C for 24hr. And then, cells were treated to TSA and incubated for 24hr at 37°C, followed by radiation. After 24 hr, cells were washed twice with PBS, and 5 mg/ml MTT in PBS was added to each well for 4 hr. After removal of the MTT solution, a solubilization solution (DMSO/EtOH, 1:1 ratio) was added to each well to dissolve the formazan crystals. The absorbance at 570 nm was measured using a ParadigmTM (Beckman Coulter).

2.2 Cell cycle analysis

The harvested cells were washed once with PBS. The pellet was fixed in 75% (v/v) ethanol for 16 hr at 4 °C. Then, cells were washed once with PBS and resuspended in cold PI solution (50 μg/ml) containing RNase A (0.1 mg/ml) in PBS (pH 7.4) for 30 min in the dark. Flow cytometry analyses were performed using FACSAriaII (Becton Dickinson).

3. RESULTS AND DISCUSSION

3.1 Changes of cell density mediated by radiation and TSA on hepatoma cells

Because radiation and TSA can modulate several biological processes including cell death, proliferation and differentiation in diverse cancers, we first examined the effects of radiation and/or TSA on morphological changes in two different hepatoma cells, HepG2 and Hep3B. As shown in Figure 1A, the density of hepatoma cells was decreased by radiation or TSA after 72 hr treatment (lanes 2 and 3) under microscopy. Moreover, the combinatorial treatment of radiation and TSA induced significant decreases in hepatoma cell density (lane 4).

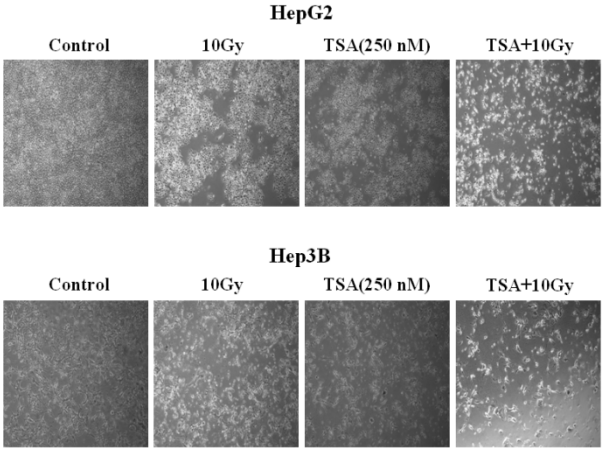

Fig. 1. Observation of HepG2 and Hep3B cell density under microscopy after 72 hr treatment of radiation and/or TSA.

3.2 Effects of radiation and TSA on hepatoma cell viability

As we observed the density changes on hepatoma cells in Figure 1, we next checked the effects of radiation and TSA on cell viability using MTT assay. When cells were exposed to radiation for 24 hr, cell viability was not changed compared to that under the normal condition in both cell lines (Fig. 2A lane 1 vs. 2 and lane 5 vs. 6). Interestingly, cell viability of HepG2 cells was significantly decreased by TSA (Fig. 2A lane 1 vs. 3), while Hep3B cell viability was not affected by TSA after 24 hr treatment (Fig. 2A lane 5 vs. 7). In addition, any synergistic effects of radiation and TSA on cell viability were not detected in both cell lines within 24 hr (Fig. 2A lanes 4 and 8). However, we observed the changes of cell viability mediated by radiation in both cell lines after 72 hr treatment of radiation (Fig. 2B lane 1 vs. 2 and lane 5 vs. 6). The cell viability of both HepG2 and Hep3B cells was also decreased by 72 hr TSA treatment (Fig. 2B lane 1 vs. 3 and lane 5 vs. 7). Although we observed the synergistic effects of radiation and TSA on cell viability in both cell lines, HepG2 cells were more sensitive to the radiation and TSA on their cell viability (Fig. 2B lanes 4 and 8). These results suggest that the sensitivity of radiation and/or TSA is different from each cell line, albeit derived from same tissue.

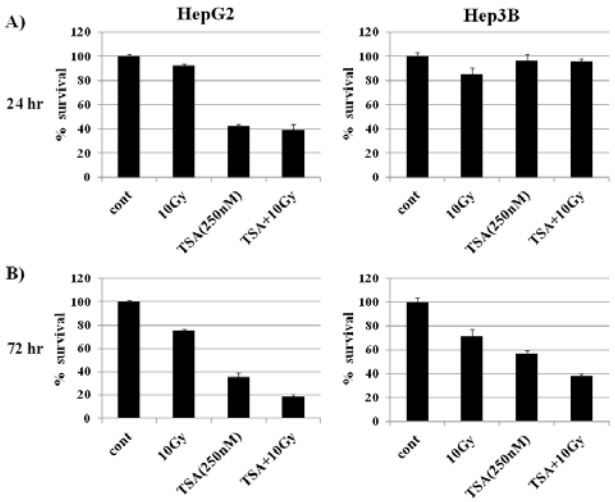

Fig. 2. Hepatoma cell viability with treatment of radiation and TSA. A) 24 hr treatment of radiation and TSA. B) 72 hr treatment of radiation and TSA.

3.3 Differential cell cycle regulation of radiation and TSA on hepatoma cells

To explore the possible mechanism of action of radiation and TSA on cell viability, we next investigated the cycle changes after treatment of radiation and/or TSA. As shown in Figure 3, with a treatment of radiation, arrested populations (G2/M phase) were significantly increased compared to that under the normal condition in both cell lines (Fig. 3, 22.9% vs. 37.2% and 24.3% vs. 35%, HepG2 and Hep3B, respectively). Unlike radiation, the treatment of TSA stimulated increases of sub-G1 populations (Fig. 3, 4.1% vs. 15.3% and 7.2% vs. 13.2%, HepG2 and Hep3B, respectively) and decreases of G1 populations (Fig. 3, 44.3% vs. 20.4% and 38.3% vs. 29.3%, HepG2 and Hep3B, respectively) in both cell lines, although arrested populations were not increased. In addition, when the

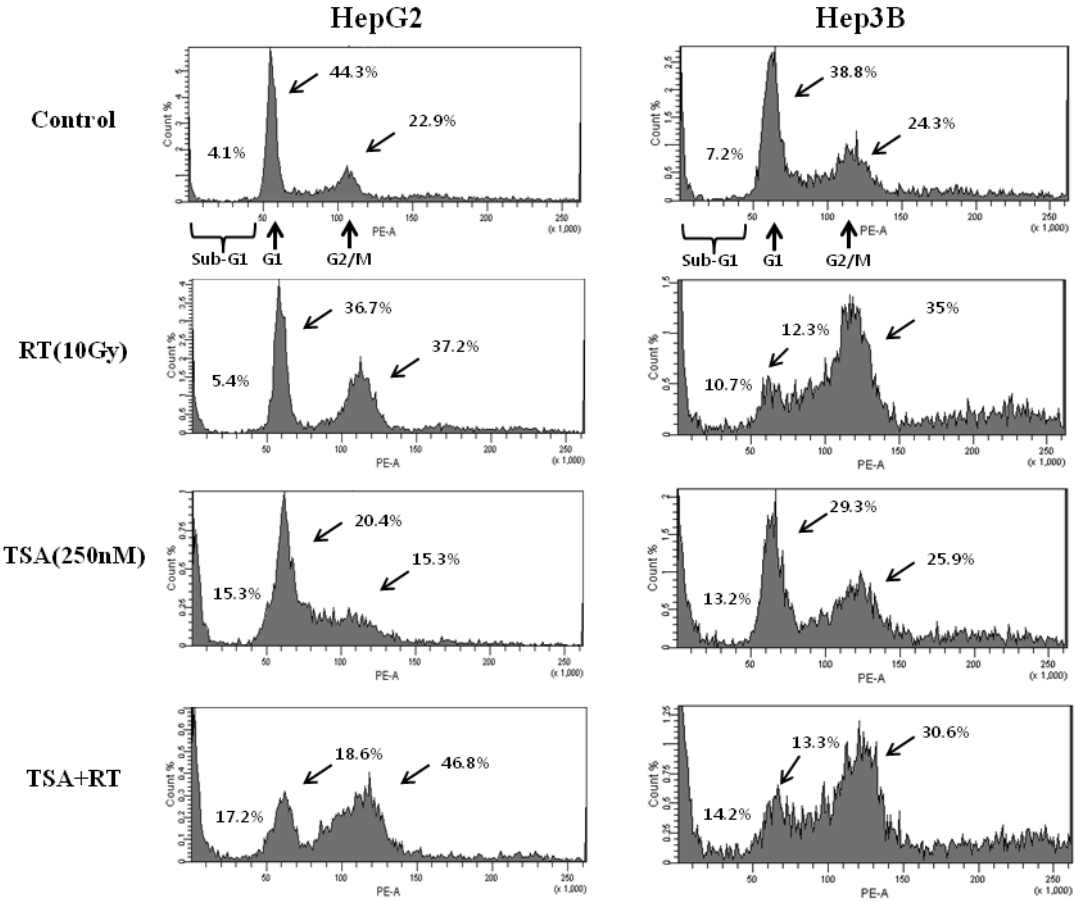

Fig. 3. Cell cycle analysis of HepG2 and Hep3B cells with treatment of radiation and TSA.

combination of radiation and hypoxia was used, death cell populations (Sub-G1) and arrested populations (G2/M) were significantly increased, whereas the G1 populations were dramatically decreased (Fig. 3, TSA+RT). Taken together, these results demonstrate that the treatment of radiation TSA induces cell cycle arrest, thereby stimulating cell death in hepatoma cells. In addition, since different cells or tissues have different reactivity to radiation and TSA, these results might be an indicator for the combination therapy with radiation and drugs in diverse cancers.

REFERENCES

[1] K. van Holde, and J. Zlatanova, "What determine the folding of the chromatin fiber?" Proc.Natl.Acad.Sci. U.S.A., vol. 93, no. 30, Oct. 1996, pp. 10548-10555.

[2] J.L. Workman, and R.E. Kingston, "Alteration of nucleosome structure as a mechanism of transcriptional regulation," Annu.Rev.Biochem., vol. 67, Jul. 1998, pp. 545-579.

[3] R.D. Kornberg, and Y. Lorch, "Twenty-five years of the nucleosome, fundamental particle of the eukaryote chromosome," Cell, vol. 98, no. 3, Aug. 1999, pp. 285-294.

[4] J.M. Espinosa, and B.M. Emerson, "Transcriptional regulation by p53 through intrinsic DNA/chromatin binding and site-directed cofactor recruitment," Mol.Cell, vol. 8, no. 1, Jul. 2001, pp. 57-69.

[5] N.A. Barlev, L. Liu, N.H. Chehab, K. Mansfield, K.G. Harris, T.D. Halazonetis, et al., "Acetylation of p53 activates transcription through recruitment of coactivators/histone acetyltransferases," Mol.Cell, vol. 8, no. 6, Dec. 2001, pp. 1243-1254.

[6] S.L. Berger, "Histone modifications in transcriptional regulation," Curr.Opin.Genet.Dev., vol. 12, no. 2, Apr. 2002, pp. 142-148.

[7] J.M. Espinosa, R.E. Verdun, and B.M. Emerson, "p53 functions through stress- and promoter-specific recruitment of transcription initiation components before and after DNA damage," Mol.Cell, vol. 12, no. 4, Oct. 2003, pp. 1015-1027.

[8] S. Nagar, L.E. Smith, and W.F. Morgan, "Characterization of a novel epigenetic effect of ionizing radiation: the death-inducing effect," Cancer Res., vol. 3, no. 2, Jan. 2003, pp. 324-328.

[9] W. An, J. Kim, and R.G. Roeder, "Ordered cooperative functions of PRMT1, p300, and CARM1 in transcriptional activation by p53," Cell, vol. 117, no. 6, Jun. 2004, pp. 735-748.

[10] A. Taddei, D. Roche, W.A. Bickmore, and G. Almouzni, “The effects of histone deacetylase inhibitors on heterochromatin: implications for anticancer therapy?” EMBO rep., vol. 6, no. 6, Jun. 2005, pp. 520-524.
[11] A. Lennartsson, and K. Ekwall, “Histone modification patterns and epigenetic codes,” Biochim.Biophys.Acta, vol. 1790, no. 9, Sep. 2009, pp. 863-868.
[12] A. Kumar, P.S. Rai, R. Upadhya, Vishwanatha, K.S. Prasada, B.S.S. Rao, et al., “γ-radiation induces cellular sensitivity and aberrant methylation in human tumor cell lines,” Int.J.Radiat.Biol., vol. 87, no. 11, Nov. 2011, pp. 1086-1096.

Sang Ho Lee
He received the B.S. in chemical engineering from Pukyung national university, Korea in 1992, the M.S. in management from Inje university, Korea in 2009 and also completed the course of a doctor degree in biology from Dong eui university, Korea in 2011. My main research interests include radiology and molecular biology.

Chang-Hee Han
He received the M.S. in fisheries biology from Pukyung national university, Korea in 1975, and Ph.D. in fisheries biology from Tokyo University, Japan in 1988. My main research interests animal physiology and molecular biology.

Su Man Kang
He acquired the M.S. in hospital political science Mister at Kosin university graduate school of public health management in 2003 and the doctorate degree in medical administration at Kosin university general graduate school of korea 2012. Current, he lectures radiology oncology in Department of Radiology, Sunrin university and Department of Radiology, Korean international university.

Woo Cheol Park
▪Graduate School of Public Health, Inje University, 2000 Master's Degree
▪2012 Department of Physics, Pukyong National University, received a Ph.D.
▪2009 to the present Department of Radiological thechnology, Dongeui institute of technology