A novel p53-activating radioresponse regulator

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1. Abstract

In order to screen ionizing radiation induced early-response genes, we employed subtractive hybridization method and isolated a metabolism associated gene. The gene expression was very sensitive to ionizing radiation as revealed by a rapid induction of its messenger RNA. We characterized the function of this gene in radiation response. This gene activated p53 and enhanced cell killing effect of ionizing radiation. This effect was attributable to p53 phosphorylation and transcriptional activation.

Key words: radiation response, p53, ATM, metabolism

2. Introduction

Cellular radiation response is mediated by ATM, a serine/threonine kinase (1). ATM performs its function by phosphorylating its protein targets such as p53, Nbs1, Chk2, and SMC1. As a result, cells arrest cell cycle, repair DNA damage or lead to cell death accordingly. One of the ATM targets, p53 is phosphorylated and increases its stability. Activated p53 protein is involved in cell cycle regulation, cell death and gene expression as a transcription factor. Therefore activation of radioresponse regulator p53 by ionizing radiation is a key step in those cellular processes following exposure to radiation.

We employed a conventional genetic screening tool to isolate p53-regulating factors and to characterize its involvement in cellular radiation response.

Our investigation isolated a gene involved in radiation response by activating p53 protein. This gene, named HSDP activates p53 when its level was increased by overexpression. P53 increase by this gene was accompanied by phosphorylation of serine 15 residue of p53 protein. Moreover increased p53 caused enhanced cell killing following exposure to ionizing radiation. Knock-down of either p53 or HSDP caused downregulation of the cell killing effect.

Therefore we report here a novel p53-activating genetic factor and its role in radiation response.

3. Material and methods

Subtractive hybridization and Gene expression:
For subtractive hybridization analysis of radiation-induced genes, we used Clontech (USA) kit and manual that accompanied the kit. The gene expression was monitored by RT-PCR analysis with beta-actin or GAPDH as control.

Cells and treatment: We used lung cancer cell line NCI-H460. For measurement of the effect of HSDP's cell viability, we stably overexpressed HSDP in the cell and treated with gamma radiation. The source of radiation is from Atomic Energy of Canada, Ltd. Cell viability was measured by clonogenic assay. Colonies with more than 50 cells were counted for clonogenic assay.

Protein methods: To determine the level of proteins we used western blot analysis using antibodies obtained from Santa Cruz, USA.

Reporter assay: To measure transcriptional activity of p53, we used p53 responsive reporter genes and performed reporter assay. This reporter contains several copies of p53 responsive sequence from p21 promoter.

4. Results and Discussion

As an initial characterization of radiation-induced genes, we performed western blot analysis of HSDP overexpressing cells (Fig. 1). In HSDP cell lines, p53 protein level increased. Accordingly, p53 target genes were activated and showed increased protein levels. These proteins included p21, Bax, MDM2. P21 and

Bax are involved in cell cycle regulation and cell death respectively. Therefore we can assume that overexpression of HSDP influences cell cycle and death by regulating upstream regulator p53. As p53 proteins are activated by phosphorylation, we examine the status p53 serine 15, a phosphorylation site of p53. As expected ser 15 was phosphorylated explaining increased stability of p53 and enhanced activity. Based on these results we conclude that HSDP increased p53's activity by enhancing its ser15 phosphorylation.

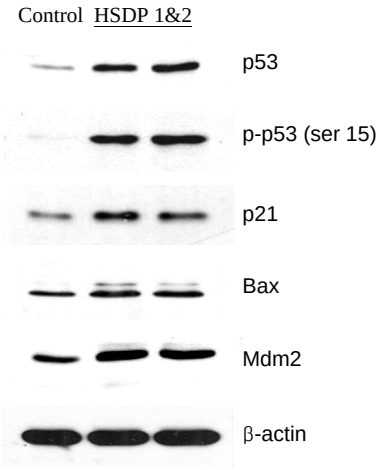

Fig. 1. Levels of p53 by HSDP.

We confirmed that the level of HSDP is proportional to p53 activity by modulating its level by SiRNA technology (Fig. 2).
Introduction of Si oligo of HSDP in the cell decreased p53 transcriptional activity as well as HSDP level. Therefore we conclude that HSDP expression is correlated with p53 level and activity.

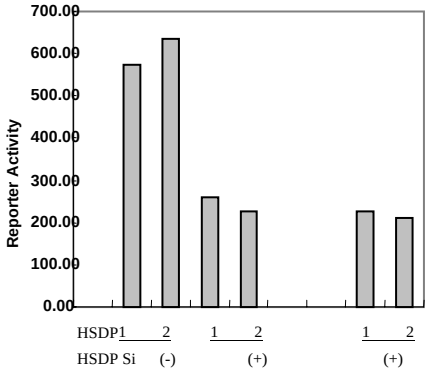

Fig. 2 HSDP knockdown experiment and its effect on p53 activity.

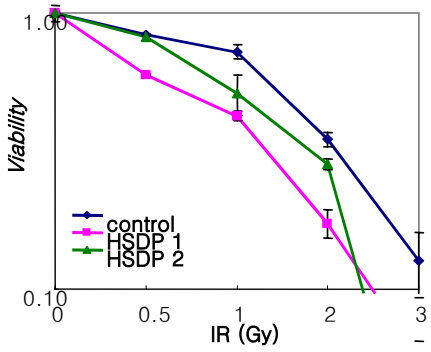

Fig. 3 Cell viability assay. Decreased colony forming ability of HSDP cells can be seen.

We next examined the effect of HSDP expression on cell viability. We used clonogenic assay to measure cell viability to ionizing radiation (Fig. 3).

Cell survival was decreased in HSDP overexpressing cells.
Taken together, correlation between HSDP and p53 activity raises the following question.

1. Does increased p53 enhance cell killing effect in HSDP cells?
2. Does HSDP directly activate P53 or use a mediator to modulate p53?

Our present work does not provide detailed answers to these questions. However we observed that p53 increases cell killing effect in HSDP cells.
As a conclusion, HSDP can modulates p53 activity by enhancing its phosphorylation. Moreover increased p53 lead to enhanced cell killing to ionizing radiation.
We wish to explore this effect to enhance radiation therapy by selectively targeting cancer cells.

Acknowledgement

This study was supported by Ministry of Science & Technology (MOST), Korean government, through its National Nuclear Technology Program.

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