A redox mechanism in the regulation of radiosensitivity by sps1

Joonmo Kim, Su-Jae Lee, Yun-Sil Lee and Sangwoo Bae*
Korea Institute of Radiological and Medical Sciences (KIRAMS)
215-4 Gongneung-Dong, Nowon-Gu, Seoul, 139-706, Korea
*Correspondence: swbae@kcch.re.kr

1. Abstract

In order to screen ionizing radiation induced early-response genes, we employed subtractive hybridization method and isolated a metabolism associated gene, sps1. Overexpression of the gene resulted in greatly reduced cellular state. A radioresponse modulator, p53 has been investigated for its possible involvement in the redox regulation by sps1. In addition to redox regulation of p53 by the reduced cellular environment, p53 transactivated several redox enzymes to affect cellular redox state. The results suggest that redox regulation of p53 might be an important factor in radiosensitivity by sps1 overexpression.

Key words: radiation response, p53, redox

2. Introduction

Cellular radiation response is mediated by DNA damage response factors (DDR factors). One of the main pathways of DDR involves ATM, Chk2 and p53ATM which modulate DNA damage repair, cell cycle regulation and cell death. ATM and Chk2 perform its function by phosphorylating its protein targets such as p53. As a result, cells arrest cell cycle or lead to cell death depending on the doses used and the degree of damage.

p53 is phosphorylated and increases its stability. Or p53 is redox activated by several chemical agents or by redox-factor-1 in the cells. Redox regulation of p53 has been suggested as a factor for cellular radiosensitivity.

We investigated sps1 for its possible involvement in p53 redox regulation and radioresponse regulation.

We employed a radiosensitive cell line NCI-H460 to overexpress sps1and found that sps1 activates p53 via redox mechanism.

3. Material and methods

Subtractive hybridization of sps1:

For subtractive hybridization analysis of radiation-induced genes, we used Clontech (USA) kit and manual that accompanied the kit. Sps1 gene clone was isolated and its sequence was confirmed.

Cells and treatment: We used lung cancer cell line NCI-H460. Stably overexpressed sps1cells were treated with gamma radiation. The source of radiation is from Atomic Energy of Canada, Ltd. Cellular redox status was measured by FACS analysis.

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.

Miscellaneous methods: please refer to the paper in the reference (1).

3. Results and Discussion

As an initial approach to elucidate sps1 activity, we measured ROS level (Figure 1). To our surprise, sps1 stable cell line exhibited less ROS compared to control cells.

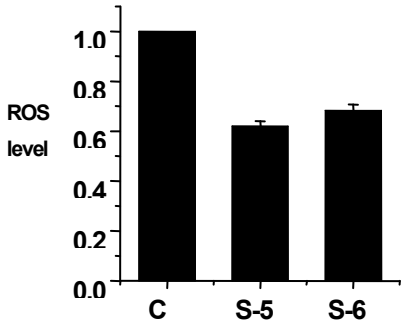

Figure 1. ROS level of sps1 cell line. C, a control cell line, S-5 and S-6, two sps1 cell lines.

To examine whether redox enzymes were differentially expressed in sps1 cell lines, several redox enzymes were examined for their total protein levels (Figure 2). Interestingly, sps1 cell lines had greater expression of MnSOD, CuZnSOD, catalase, Trx while cGPX was decreased.

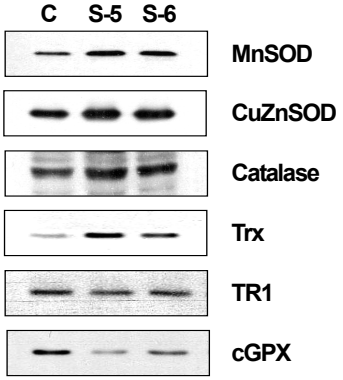

Figure 2. Redox enzyme levels.

Since we expect that sps1 overexpression and its modulator p53 is involved in the regulation of the redox enzymes, p53 level was modulated to test its involvement (Figure 3). Upon depletion of p53 by Si RNA, Trx, CuZnSOD, and MnSOD were decreased while catalase was unchanged.

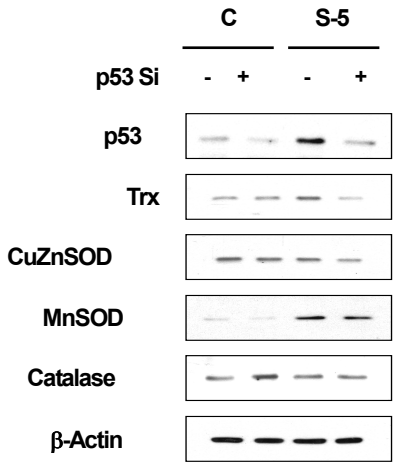

Figure 3. Effect of p53 on the expression of the redox enzymes.

Based on the results, we conclude that sps1 overexpression results in reduced cellular environment and in greatly increased expression of redox enzymes.

We also expected the possibility that the reduced cellular environment affect p53's activity (Figure 4).

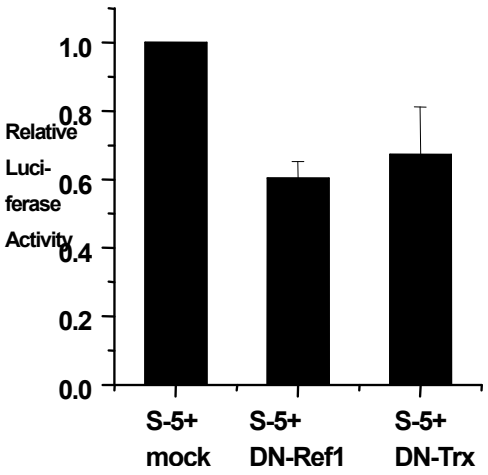

Figure 4. Redox regulation of p53 by Ref1. Reporter assay for p53 transcritional activity.

P53 activity as a transcription factor was increased by the reduced environment. Ref-1 (redox factor-1) was involved in the regulation. Thus sps1 overexpression leads to p53 activation and this in turn elevates cellular redox enzyme expression. Redox elevation further enhance p53 activity via Ref1. Since p53 is a critical factor in DDR, sps1 activity in the cell might determine radiosensitivity via p53-mediated redox regulation.

Acknowledgement

This study was supported by Ministry of Science & Technology (MOST), Korean government, through its National Nuclear Technology Program. This study and other related results have been published (1).

Reference

1. Chung et al. J Cell Physiol. 2006 Oct;209(1):131-41. p53-mediated enhancement of radiosensitivity by selenophosphate synthetase 1 overexpression.