Involvement of p27CIP/KIP in HSP25 or HSP70 Mediated Adaptive Response by Low Dose Radiation

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1. Introduction

Adaptive responses that reduce the harmful effects of subsequent exposure to high-dose radiation have demonstrated in chromosome aberration, cell survival, sister chromatid exchanges, micronucleus induction, mutation and neoplastic transformation. The mechanisms and conditions for the adaptive response to radiation have not been clarified, although the continuous production of free radicals from radiation and other sources has stimulated cells to evolve a repair system for chromosome breaks. An alteration of the DNA molecule triggers the repair system, and frequent activation may increase the general repair capacity, irrespective of the cause of the damage. Besides, cell cycle regulation systems, antioxidant defense systems, molecular chaperone or stress-response systems. Our previous data showed that when cells were preirradiated with 1cGy, they showed the adaptive response. A reduction of apoptosis by low-dose preirradiation is another potential mechanism for this effect (1).

We previously demonstrated that mouse RIF cells, which did not induce HSP25 and HSP70 did not exhibit a adaptive response after 1cGy preirradiation (2). whereas the thermoresistant TR cells, which expressed inducible HSP25 and HSP70 showed a response. Moreover, when HSP70 and HSP25 were transfected to RIF cells, the cells acquired adaptive response. In this study, to elucidate the mechanisms in induction of adaptiveresponse, we compared cell cycle distribution by low dose radiation after HSP25 or HSP70 transfected cells and p27CIP/KIP is responsible for the different induction of adaptive response.

2. Materials and Methods

Cell culture

RIF (radiation-induced fibrosarcoma cells), and TR (a thermoresistant clone of RIF) were cultured in Dulbecco's minimal essential medium supplemented with heat-inactivated 10% fetal bovine serum and antibiotics at 37℃ in a 5% CO2 humidified incubator.

Flow cytometric analysis

Cells were cultured, harvested at the indicated times, stained with propidium iodide, according to the manufacturer's protocol, and then analyzed using a FACScan flow cytometer

Polyacrylamide gel electrophoresis and Western blot

For polyacrylamide gel electrophoresis (PAGE) and Western blot, cells were solubilized with lysis buffer, the samples were boiled for 5 min, and equal amount of protein (40 mg/well) was analyzed on 10% SDS-PAGE. After electrophoresis, proteins were transferred onto a nitrocellulose membrane and processed for immunoblotting. Blots were further incubated with horseradish peroxidase-conjugated secondary antibody, diluted at 1:5,000, and specific bands were visualized by chemiluminescence. Autoradiographs were recorded onto X-Omat AR films.

3. Results

HSP25 and HSP70 involves in adaptive response by low dose radiation

When cells were preirradiated with 1cGy before a high challenging dose of radiation, adaptive

responsewas examined in TR cells, but not in the parental RIF cells. By nocodazole treatment before radiation, mitotic cell arrest which is a typical radiation induced cell cycle arrest and these cells usually go to cell death, decreased mitotic arrest was shown in low dose preirradiated cells with 4 hr interval between low and high challenging irradiation. Since the expression of HSP25 and inducible HSP70 have been shown to be different in these two cell lines , HSP25 and inducible HSP70 were transfected to RIF cells and determined whether there was any link between HSPs and the induction of an adaptive response. Clonogenic cell survival assay revealed that HSP25 or inducible HSP70 overexpression acquired adaptive response whereas, their parent RIF cells did not show an adaptive response. When induction of apoptosis was examined, similar phenomena was observed.

Increased G1 arrest by low dose radiation is shown by HSP25 and inducible HSP70

To examine the mechanisms of induction of adaptive response, cell cycle distribution was checked after 1cGy low dose radiation Increased G1 arrest was observed in TR,HSP25 and inducible HSP70 overexpressing cells. Thymidine incorporation data also suggested that HSP25 and inducible HSP70 involved in the induction of adaptiveresponse. To examine the mechanisms, we examined expression levels of proteins which are related to G1 arrest. Expression of p27CIP/KIP was increased by HSP25 and inducible HSP70 and antisense treatment of p27 abolished G1 arrest by low dose radiation in HSP25 and inducible HSP70 overexpressed cell.

4. Conclusion

HSP25 and inducible HSP70 were responsible for the induction of an adaptive response and increased expression of p27CIP/KIP may be a key modulator of this phenomena.

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