Molecular Targets for Radioprotection by Low Dose Radiation Exposure

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Adaptive response is a reduced effect from a higher challenging dose of a stressor after a smaller inducing dose had been applied a few hrs earlier. Radiation induced fibrosarcoma (RIF) cells did not show such an adaptive response, i.e. a reduced effect from a higher challenging dose (2 Gy) of a radiation after a priming dose (1 cGy) had been applied 4 or 7 hrs earlier, but its thermoresistant clone (TR) did. Since inducible HSP70 and HSP25 expressions were different between these two cell lines, the role of inducible HSP70 and HSP25 in adaptive response was examined. When inducible *hsp70* or *hsp25* genes were transfected to RIF cells, radioresistance in clonogenic survival and reduction of apoptosis was detected. The adaptive response was also acquired in these two cell lines, and inducible *hsp*70 transfectant showed more pronounced adaptive response than *hsp25* transfectant. From these results, inducible HSP70 and HSP25 are at least partly responsible for the induction of adaptive response in these cells. Moreover, when inducible HSP70 or HSP25 genes were transfected to RIF cells, co-regulation of each gene was detected and heat shock factor (HSF) was found to be responsible for these phenomena.

In continuation of our earlier study on the involvement of heat shock protein (HSP) 25 and

HSP70 in the induction of adaptive response, we have now examined the involvement of these proteins in the induction of the adaptive response, using an animal model system. C57BL6 mice were irradiated with 5 cGy of gamma radiation 3 times for a week (total of 15cGy) and a high challenge dose (6Gy) was given on the day following the last low dose irradiation. Survival rate of the low dose pre-irradiated mice was increased to 30%. Moreover, high dose-mediated induction of apoptosis was also reduced by low dose pre-irradiation. To elucidate any link existing between HSP and induction of the adaptive response, reverse transcriptase (RT)-polymerase chain reaction (PCR) analysis was performed using splenocytes. High dose radiation up-regulated the expression of HSP25 and especially HSP70; while expression of other HSPs such as HSC70, HSP90, and αB-crystalline did not change. When splenocytes from HSP70 transgenic mice were pre-irradiated with a low dose of radiation, a reduction in cell death by high dose radiation was observed. These results, suggest that HSP70 is a key molecule in radioprotective effect by low dose radiation.

Introduction

Many studies have shown that low doses of ionizing radiation can produce a stimulatory effect and induced adaptive responses to the harmful effects of subsequent high-dose radiation exposure. Several recent studies have shown that adaptive responses were observed in chromosome aberrations, cell survival, sister chromatid exchanges, micronuclei, mutation and neoplastic transformation. The mechanisms of, and conditions for, adaptive response to radiation have not been clarified, though one possible explanation relates to the induction of DNA repair processes in response to low-doses of around 0.01 Gy. The induction of new proteins in response to low-doses is regarded as experimental support for this explanation. Our previous data showed that normal cells showed the adaptive response, when 1 cGy was

preirradiated, but neoplastic cells did not. Reduced apoptosis by low dose preirradiaiton is another mechanisms for this effect. This adaptive response seems to be most prevalent in radioresistant cell lines, since several sensitive cell lines failed to show the increase in radioresistance beyond 0.5 Gy.

It is well established that members of the HSP family function as molecular chaperones and assist in the intracellular folding of newly synthesized of denatured proteins. Several investigators have reported the induction of a HSP70 member protein during the adaptive response to oxidative stress with H_2O_2. This induction was found to occur during the pretreatment of cells with a low concentration of H_2O_2. Low doses of X-rays on the activation of the promoter of the human heat-shock protein HSP70B gene, whose transcription is silent in control conditions but is highly inducible by heat shock treatment. Low dose of 4 cGy radiation, which induced adaptive response, increased HSP70 mRNA. In addition, induction of PBP74/mortalin/Grp75, a member of the HSP70 family, by low dose of ionizing radiation also involved in radioadaptive response. In the present study, we demonstrated inducible HSP70 and HSP25 are important for the induction of the adaptive response and radioprotective effects.

Results

Adaptive response was detected in TR, while it was not in its parental RIF cells.

When 0.01 Gy was pre-irradiated before a high challenging dose of radiation, adaptive response was detected in TR cells, while it was not in its parental RIF cells. Since there is a report that HSP expression profile is different between two cell lines, we analyzed these protein expression by western blotting. Inducible HSP70 and HSP25 expression was dramatically increased in TR cells, even though slight induction of HSP90 and truncated form of HSP110 were found in TR cells, suggesting that adaptive response is related with these protein expressions. Irradiation with 0.01 Gy or 4 Gy did not change the HSP protein expressions in this

system.

Overexpression of inducible HSP70 and HSP25 was regulated each other.

First, to examine the role of HSP25 and inducible HSP70 on the induction of adaptive response, inducible HSP70 or HSP25 was transfected to RIF cells in which the adaptive response was not induced. When HSP25 was overexpressed, increased expression of inducible HSP70 was found, and more, when inducible HSP70 was overexpressed, increased induction of HSP25 was detected. To know if these expressions were regulated in transcription level, PCR analysis for HSP25 and inducible HSP70 were performed. Increased HSP25 mRNA was detected in HSP25 transfectant cells with increased level of inducible HSP70. In addition, inducible HSP70 mRNA was also detected in inducible HSP70 transfectants as well as increased HSP25 mRNA level. When we transfected HSP25 or inducible HSP70 to L929 cells or NIH3T3 cells, these phenomena were not detected.

Inducible HSP70 and HSP25 induced radio-resistance and reduced radiation-induced apoptosis

Overexpression of HSP25 or inducible HSP70 induced radio-resistance when clonogenic survival was used; the tendency of radio-resistance was similar in inducible HSP70 and HSP25 transfectants. Nuclear staining using Hoechst 33254 also revealed that radiation induced-apoptosis was decreased by 10-20% of control vector cells; the tendency was higher in inducible HSP70 than HSP25 transfectant cells, suggesting inducible HSP70 and HSP25 are responsible for the radio-resistance.

Adaptive response was induced in inducible HSP70 or HSP25 transfectant RIF cells

To know the molecule that is responsible for the induction of adaptive response, adaptive response was examined between HPS25 and inducible HSP70 transfectant cells. In clonogenic survival assay, adaptive response was found in both inducible HSP70 and HSP25 transfectant

cells and more increased effect was found in inducible HSP70 transfectant cells. Similarly, when 0.01 Gy was preirradiated, reduced apoptosis was found in both inducible HSP70 and HSP25 transfectant cells when compared to the high challenging dose alone treated cells; this effect was also predominant in inducible HSP70 than HSP25 transfectant cells.

Discussions

The present study represents that mouse RIF cells in which inducible HSP70 and HSP25 are not expressed, did not show the adaptive response by 1 cGy of low dose pre-irradiation, while its thermoresistant cells of TR in which inducible HSP70 and HSP25 are expressed, did. In addition, when inducible HSP70 or HSP25 were transfected to RIF cells, the radioresistance was gained and radioadaptive response was expressed; inducible HSP70 transfectant cells showed more increased induction of adaptive response, suggesting that inducible HSP70 is important for the induction of the adaptive response and radioresistance.

Radioadaptive response, first described by Olivieri et al in 1984 in cultured human lymphocytes and later confirmed by others in a wide variety of animal and plant cells, has been conformed by several characteristics: (a) the adaptation is a rapid process, being fully expressed 4-6 hr after irradiation, and continues for more than 20 hrs; and (b) it has a dose limitation for an optimal expression below ~0.1 Gy and higher doses are not only incapable of inducing adaptation but also rapidly erase the adapted state which has been previously induced by lower doses. However, the molecular mechanism and signaling pathway effecting the regulation of such a response are remained unsolved.

In our results, HSP25 overexpression induced the expression of inducible HSP70 protein and inducible HSP70 overexpression also induced HSP25 protein. These expressions were transcriptionally regulated each other. We do not know how these two proteins regulated each

other and what is the major factor for those. When we transfected inudcible HSP70 or HSP25 to NIH3T3 or L929 cells, these phenomena was not found. So, these might be unique effect in RIF cells. Further study is being on the progress, now.

There are reports that low dose-irradiated cells showed many responses by synthesizing some protein such as HSPs. In our system, HSP expression was not detected by Western blotting, however, one possible reason was considered for no detectable HSPs (especially inducible HSP70 and HSP25) expression by radiation: the expression of HSP by radiation was so low as to be undetectable. The increase of HSP70 was reported to be detected in Chinese hamster ovary cells exposed to 400 or 1000 Gy. Therefore, we transfected inducible HSP70 and HSP25 to RIF cells, which did not induced these proteins and did not show adaptive response, and these transfectant cells required the adaptive response. From these results, inducible HSP70 and HSP25 were responsible for the induction of adaptive response.

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