Protective Effect of HSP25 on Radiation Induced Tissue Damage

Hae-June Lee[1], Yoon-Jin Lee[1], Hee-Choong Kwon[2], Sangwoo Bae[1], Sung-Ho Kim[3] and Yun-Sil Lee[1]
[1]*Laboratory of Radiation Effect,* [2]*Laboratory of Molecular Oncology, Korea Institute of Radiological Medical Sciences, 215-4 Gongneung-Dong, Nowonk-Ku, Seoul, 139-706, Korea, and* [3]*College of Veterinary Medicine, Chonnam National University, 300 Yongbong-Dong, Puk-Ku, Gwangju, 500-757, Korea*

1. Introduction

Control of cancer by irradiation therapy alone or in conjunction with combination chemotherapy is often limited by organ specific toxicity. Ionizing irradiation toxicity is initiated by damage to normal tissue near the tumor target and within the transit volume of radiotherapy beams. Irradiation-induced cellular, tissue, and organ damage is mediated by acute effects, which can be dose limiting. A latent period follows recovery from the acute reaction, then chronic irradiation fibrosis (late effects) pose a second cause of organ failure. HSP25/27 has been suggested to protect cells against apoptotic cell death triggered by hyperthermia, ionizing radiation, oxidative stress, Fas ligand, and cytotoxic drugs. And several mechanisms have been proposed to account for HSP27-mediated apoptotic protection [1-3]. However radioprotective effect of HSP25/27 *in vivo* system has not yet been evaluated. The aim of this study was to evaluate the potential of exogenous HSP25 expression, as delivered by adenoviral vectors, to protect animal from radiation induced tissue damage.

2. Materials and Methods

Animals and irradiation

Adenoviral vectors ($1x10^8$ pfu /head) including HSP25 or pcDNA were delivered to submandibular gland of Sprague Dawley rats directly or bone marrow of C57BL/6 mice by intravenous injection at 24hrs before irradiation. And amifostine (100 mg/kg) were administrated intravenously at 30 mins before irradition as a positive control. Experimental mice were performed whole body irradiation with 4.5 Gy and ventral head and neck region of rats exposed to 17.5 Gy from a ^{60}Co gamma-ray source.

Sample analysis

Bone marrow samples obtained at 1, 4, 7, 14, and 21 days following IR. At each endpoint blood count was performed. Right femurs examined general hitopathology, cellurarity, and immunohistochemistry. And bone marrow cells of left femurs were used in immunoblotting.

To investigate effect of HSP25 on salivary glands, saliva secretions by pliocarpine and biochemical analysis of saliva were performed. Salivary glands were tested using immunoblotting or histopatholgical analysis at 40 and 90 days after IR.

3. Results

Effect of HSP25 gene transfer on submadibular glands

At 40 and 90 days after IR treatment (17.5 Gy) of salivary gland, there was significant reduction of salivary output respectively. However, gene transfer of HSP25 dramatically increased saliva secretion up to 70~80% of control level (Table 1). We measured Aquaporin5 expression level by immunohistochistry and there were more AQP5 positive aicnar cells in HSP25 transferred rats than the control vector transferred glands (Fig. 1). And also apoptotic cell death was significantly decreased in HSP25 gene transfer group. These results indicate that HSP25 is a good candidate molecule to protect salivary gland from the toxicity of irradiation.

Table 1. Salivary flow rate stimulated by pilocarpine

Groups	Salivary flow rate (μl/30min/100g body weight)	
	40 days	90 days
Normal control	20.99±2.18	21.35±3.15
Vector control	21.40±5.03	17.2±1.13[a]
HSP25	17.63±5.69	21.73±10.3
IR control	9.97±2.28	5.19±3.53[a]
Vector + IR	7.27±0.75[b]	6.61±4.70[b]
HSP25 + IR	15.21±4.03[c]	15.21±4.03[c]
Amifostine + IR	13.65±0.61[d]	16.39±3.42[d]

[a, b, c, d] denote statistical significance of $p<0.05$

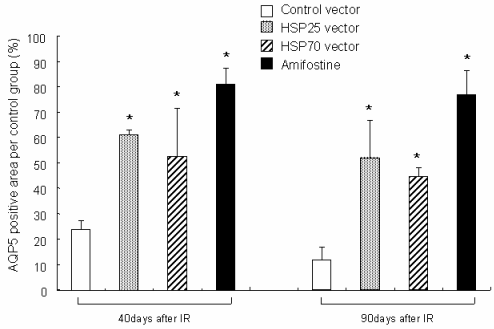

Figure 2. Distribution of AQP5 in salivary glands shows radioprotective effect of HSP25 and amifostine treatment against gamma-ray irradiation. * denotes significance compared with control group ($p<0.05$).

Effect of HSP25 gene transfer to bone marrow

After irradiation in mice, bone marrow HSCs were significantly decreased and slowly reconstructed. At

histopathological analysis, HSP25 showed rapider restored of HSCs (Fig. 2) and less apoptosis than control viral vector administrated group during postirradiational days. These results indicated that the administration of HSP25 to mice leads to significant recovery of tissue damage hematopoiesis and protection of radiation induced damage.

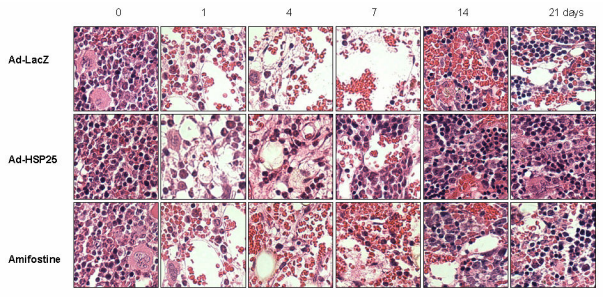

Figure 2. Recovery HSCs in bone marrow during post irradiation days

4. Conclusion

Our studies have shown that HSP25 administration has a function of recovery and protection in radiation induced damage *in vivo*. These results suggested that HSP25 could be used as a therapeutic protein for specific protection against radiation induced tissue injury

References

[1] A. Samali and T. G. Cotter. Heat shock proteins increase resistance to apoptosis. Exp Cell Res, Vol. 223, p163, 1996.

[2] Y. J. Lee, H. N. Cho, D. Jeoung, J. W. Soh, C. K. Cho, S. Bae, H. Y. Chung, S. J. Lee and Y. S. Lee. HSP25 overexpression attenuates oxidative stress-induced apoptosis: roles of ERK1/2 signaling and manganese superoxide dismutase. Free Radic Biol Med, Vol. 34, p429, 2004.

[3] Y. J. Lee, D. ,H. Lee C. K. Cho, S. J. Lee, J. W. Soh and Y. S. Lee. HSP25 inhibits protein kinase C delta-mediated cell death through direct interaction. J Biol Chem, Vol. 280, p18108, 2005.