HSP25 Protects Radiation-Induced Salivary Gland Damage

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

1. Introduction

Irradiation (IR) is a central treatment modality administered for head and neck malignancies. A significant consequence of this IR treatment is irreversible damage to salivary gland in the IR field. While the exact mechanism of salivary gland damage remains enigmatic, fluid secreting acinar cells are lost, and saliva output is dramatically reduced [1-2]. Previously we have reported that heat shock protein 25 (HSP25) induced radioresistance *in vitro* [3]. HSP25 interferes negatively with apoptosis through several pathways which involve its direct interaction with cytochrome c, protein kinase c delta or Akt. And localized gene transfer to salivary glands has great potential for the treatment of salivary gland [4]. Herein, we investigated whether HSP25 can use as radio protective molecules for radiation-induced salivary gland damage *in vivo.*

2. Methods and Results

Animals

Male Wistar rats (250-300g) were purchased from Jung-Ang lab animal Inc. and were kept under in conventional condition with free access to water and food.

Irradiation and gene transfer

Experimental rats were subjected to *in vivo* adenoviral-mediated gene transfer ($1x10^9$ pfu of HSP25) to the submandibular gland. 24 hours after gene transfer, rats were irradiated with a single dose of 17.5 Gy, with ventral surface of their head and neck exposed to the source.

Clinical laboratory and histological analyses

Samples obtained at 40 days and 12 weeks following IR. Salivary flow rates and volume were determined on anesthetized animal following an intramuscular injection of pilocarpine (0.1mg/kg). Saliva and blood were analyzed by standard clinical chemistry and hematology procedures. Tissue blocks of irradiated salivary glands were fixed in 4% formalin, embedded in paraffin, and sectioned at 3-4 ㎛. The sections were either with hematoxylin and eosin, examined for evidence of pathological changes, or processed for immunohistochemical detection of TUNEL, PCNA, aquaporin 5 (AQ5) and HSP25.

3. Results

At 40 days and 12 weeks after IR treatment (17.5 Gy) of salivary gland, there were >65% and >72% reduction in salivary output respectively. Salivary flow rate in normal animals was 20.99 ± 2.18 ㎕/min/100g body weight, while the flow rate of IR treated rats were 7.27 ± 0.75 ㎕/min/100g body weight (in 40 days) and 6.61 ± 4.70 ㎕/min/100g body weight (in 12 weeks). However, salivary output in HSP25 treated rats was significant increased to 15.21 ± 4.03 (in 40days) and 14.17 ± 0.85 ㎕/min/100g body weight (in 12 weeks). Concentrations of amylase, total protein and calcium ion in saliva were also elevated in HSP25 transferred groups. We measured HSP25 protein expression level by immunohistochistry and there were an increase in HSP25 positive aicnar cells in HSP25 transferred rats, when compared to the control vector transferred glands.

These results indicated that the administration of HSP25 to the salivary glands leads to significant recovery of secretary function and to protection of radiation induced damage.

Table. Salivary chemistry and flow rate at 40days and 12 weeks following IR (17.5 Gy)

	Flow rate (㎕/min/100g)	Amylase (u/L)	Total protein (mg/dL)	Ca^{2+} (mM/L)
Control	20.99±2.18	62650±15450	9.66±5.03	0.35±0.10
40 days				
Vector+IR	7.27±0.75	53568±6670	7.33±1.53	0.31±0.13
HSP25	17.63±5.69	70613±10394	13.0±2.65	0.60±0.08
HSP25+IR	15.21±4.03**	72320±12979**	13.67±1.53*	0.39±0.09
12 weeks				
Vector+IR	6.61±4.70	38100±12000	35.67±19.4	0.32±0.07
HSP25	21.73±10.3	58333±26383	52.3±20.9	0.43±0.02
HSP25+IR	14.17±0.85*	67500±47168*	53.0±28.3	0.43±5.30**

*$p<0.01$, **$p<0.05$ as compare with viralvector transfer groups

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 might use as a radioprotective molecule in radiation therapy of head and neck.

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