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Inducible HSP70 Protects Radiation-Induced Salivary Gland Damage

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1. Introduction

Irradiation (IR) delivered to the head and neck is a common treatment for malignancies. Salivary glands in the irradiation field are severely damaged, and consequently this resulted in marked salivary hypofunction. 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 inducible heat shock protein 70 (HSP70i) induced radioresistance *in vitro* [3-4]. Moreover, HSP70i localized to salivary glands by gene transfer has great potential for the treatment of salivary gland [5]. Herein, we investigated whether HSP70 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 SCL and were kept under in conventional condition with free access to water and food. Experimental animals were randomly divided 7 groups; normal control, radiation control, vector control, vector irradiation control, HSP70 transferred group, HSP70 transferred and irradiation group, and Amifostine (positive control) group.

Irradiation and gene transfer

Rats were subjected to *in vivo* adenoviral-mediated HSP70 transfer ($1x10^8$ pfu /gland) to the submandibular gland at 24 hrs before irradiation. Amifostine was treated at 30 mins before irradiation (100 mg/kg/body weight, *i.v.*) Animals 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 90 days 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 (AQP5) and HSP70.

3. Results

At 40 days and 90 days after IR treatment (17.5 Gy) of salivary gland, salivary secretion was dramatically reduced by radiation, compared to unirradiated control rats. However, salivary output in HSP70i or amifostine treated rats was significant ameliorated (Table 1). Histopathologic changes of salivary glands, vacuolization, pyknotic nuclei, and fibrosis, were attenuated by HSP70i or amifostine. In immunohistochemistry, AQP5 was located at apical membrane of secretory cells and AQP5 positive cells are abundant in non-irradiated salivary glands. 17.5 Gy of radiation significantly reduced these expressions from 40 days and at 90 days, almost no AQP5 was presented. However, HSP70i and amifostine protected radiation induced reduction of AQP5 expression (Figure 1). Radiation induced apoptosis of acinar cell, intercalated ductal cell, and convoluted granular cell was also significantly reduced by HSP70i or amifostine treatment at 40 or 90 days after IR.

These results indicated that the administration of HSP70i to the salivary glands leads to significant recovery of secretary function and to protection of radiation induced damage.

Table 1. Salivary flow rate stimulated by pilocarpine of the different groups of rats

Groups	Saliva flow rate (㎕/30min/100g B.W)	
	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]
HSP70i	23.07±3.04	15.56±11.77
Radiation control	9.97±2.28	5.19±3.53[a]
Vector + IR	7.27±0.75[b]	6.61±4.70[b]
HSP70i + IR	12.04±4.99[c]	12.58±2.59[c]
Amifostine + IR	13.65±0.61[d]	16.39±3.42[d]

Values are mean±SD of 3 rats per groups.
[a]; significant different form the normal control group
[b]; significant different form the vector control group
[c]; significant different form the vector transferred irradiation control group
[d]; significant different form the irradiation control group
[a, b, c, d] denote statistical significance of $p<0.05$

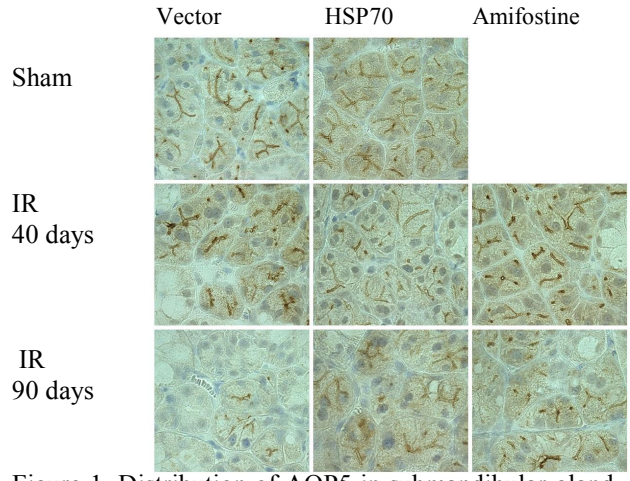

Figure 1. Distribution of AQP5 in submandibular gland at 40 and 90 days after irradiation. HSP70 transfer and amifostine administration significantly inhibited AQP5 reduction.

4. Conclusion

Our studies have shown that HSP70i administration has a function of recovery and protection in radiation induced damage *in vivo*. These results suggested that HSP70i might use as a radioprotective molecule in radiation therapy of head and neck.

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References

[1] R. M. Nagler. The enigmatic mechanism of irradiation-induced damage to the major salivary glands. Oral diseases, Vol.8, p.141, 2002.

[2] R. M. Nagler, B. J. Baum, G. Miller and P. C. Fox, Long term salivary effects of single dose head and neck irradiation in the rat. Archives Oral Biology, Vol.43, p.297, 1998.

[3] S. H. Park, S. J. Lee, H. Y. Chung, T. H. Kim, C. K. Cho, S. Y. Yoo and Y. S. Lee. Inducible heat-shock protein 70 is involved in the radioadaptive reaponse, Radiation research, Vol.153, p318, 2000.

[4] S. J. Lee, S. A. Choi, K. H. Lee, H. Y. Chung, T. H. Kim, C. K. Cho and Y. S. Lee. Role of inducible heat shock protein in radiation-induced cell death, Cell Stress Chaperones, Vol. 6, p.273, 2001.

[5] J. M. Vitolo and B. J. Baum. The use of gene transfer for the protection and repair of salivary gland, Oral diseases, Vol.8, p.183, 2002.