Identification of radioresistance-related molecules in laryngeal cancer cells using proteomic and EST data mining approach

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1. Introduction

Laryngeal cancer is the largest subgroup of head and neck cancer which is the sixth most prevalent cancer in the world [1]. Radiotherapy is known as a major treatment modality of laryngeal caner in conjunction with surgery and chemotherapy. Clinical radiotherapy is generally based on the treatment of fractionated radiation (commonly 2 Gy daily to total 60-70 Gy) to the cancer. This chronic treatment can trigger tumor-adaptive radioresistance contributing cancer recurrence following radiotherapy [2]. Unfortunately, approximately 15 % of laryngeal cancers after radiotherapy acquire radioresistance [3]. However, little is known about the molecular markers and mechanisms underlying tumor-adaptive radioresistance. In the present study, we established the radioresistant model system using HEp-2 cell line and identified radioresistance-related molecules by using the analysis of laryngeal cancer expressed sequence tag (EST) data bases and two-dimensional polyacrylamide gel electrophoresis (2D-PAGE).

2. Methods and Results

To investigate molecular markers and mechanisms of radioresistance for laryngeal cancer, radioresistant laryngeal cancer cells (HEp-2) were established by chronic treatment of fractionated radiation. Candidates of radioresistance-molecules were identified by using proteomic and EST data mining tools. For EST data mining, public EST database of laryngeal cancer (Lib. 992, 6910, 6653, 6655, 6915, 9282, 7793) were used. Several biological experiments including clonogenic, cell proliferation, apoptosis assay, and RT-PCR analysis were carried out to characterize the established radioresistant HEp-2 cells.

2.1 Establishment and characterization of radioresistant laryngeal cancer Hep-2 cells

In order to develop radioresistant HEp-2 cells, cells were irradiated twice per a week for a total 60 Gy of γ-radiation. Clonogenic assay was shown that irradiated HEp-2 cell population was a more radioresistant than parental HEp-2 cells in dose-dependent manner (Fig. 1). Thus, the irradiated HEp-2 cells were named as radioresistant HEp-2 cells (RR-HEp-2 cells). In addition, variant subclones were isolated because of heterogeneity of the irradiated HEp-2 cell population. Out of isolated subclones, RR-#6 clone was used as the representative radioresistant HEp-2 cells.

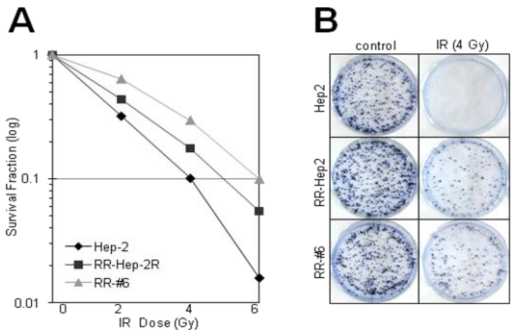

Fig. 1. Different radioresistance of parental HEp-2 cells, irradiated HEp-2 cells (RR-HEp-2) and it's variant clone (RR-#6).

Next, we determined the rate of cell proliferation and apoptosis by ionizing radiation in parental HEp-2 and RR-HEp-2 cells. As shown in Fig 2A, RR-HEp-2 and RR-#6 cells have more cell proliferative activity than parental HEp-2 cells under sham or γ-radiation (4 Gy) treatment indicating the established RR-HEp-2 cells can overcome growth inhibitory effects by ionizing radiation. In addition, radiation-induced apoptosis was suppressed in RR-HEp-2 and RR-clones (#6 and#13) as determined by Annexin-V apoptosis assay (Fig. 2B). Taken together, above results clearly indicate that the established HEp-2 cells represent the radioresistant phenotype having general physiological characteristics of radioresistant cancer cells.

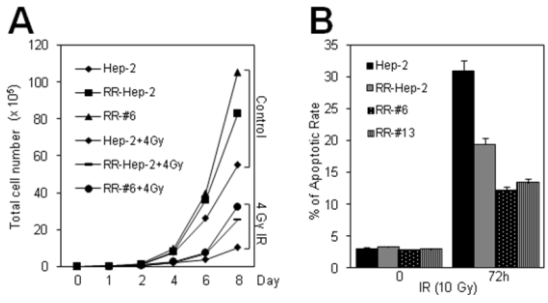

Fig. 2. Cell proliferation and apoptosis analysis of parental HEp-2, RR-HEp-2, and its variants by ionizing radiation (clone #6 and #13).

2.2 Identification of radioresistantce-related genes using EST data mining

The EST data base provides valuable information on regulatory genes displaying transcriptional expression profiles [4]. We analyzed six laryngeal cancer UniGene

libraries. Out of 8,427 clustered ESTs from the libraries, 1,729 genes were predicted as the genes encoding a protein. Using bioinformatics category selection for the regulatory molecules of cell cycle, proliferation, apoptosis, and several regulatory molecules, 68 genes were finally selected as candidate radioresistance-related molecules. Radiation-responsive genes such as p21, Bcl-xl, HSPs, and EGFR family are involved in radiation resistance and sensitivity [5]. Therefore, base on 68 candidate genes (designated as L1-68), 16 radiation-responsive genes were identified in which expressions are modulated as time-dependent manner in γ-radiation treatment to HEp-2 cells (Fig. 3A). Out of 16 radiation-responsive genes, we finally identified 8 radioresistance-related genes (middle line) using the analysis of differentially expression pattern of candidate genes between parental HEp-2 cells and radioresistant HEp-2 cells (RR-HEp-2 and RR-#6) (Fig. 3B).

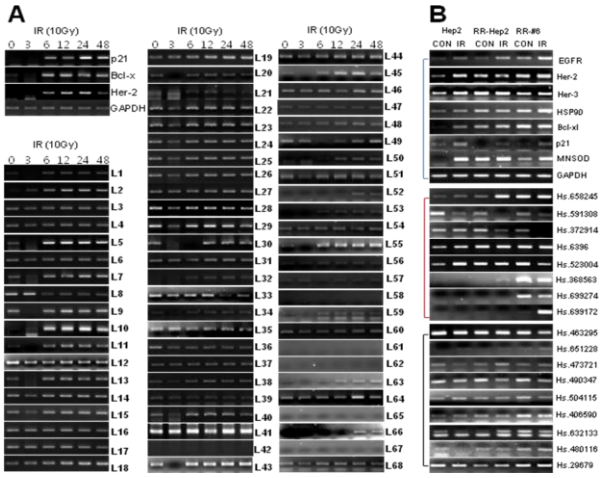

Fig. 3. RT-PCR screening and expression analysis of candidate radioresistance-genes selected from six laryngeal cancer EST data base.

2.3 Identification of radioresistantce-related proteins using proteomic approach

To identify differentially regulated proteins in the radioresistant HEp-2 cells, we next analyzed global protein expression profiles between parental HEp-2 and radioresistant HEp-2 cells by utilizing 2D-PAGE. Protein spots in 2D-PAGE gels were visualized by silver-staining method. By gel image analysis, 20 protein spots were identified as differentially expressed proteins with the significance (Fig. 4). 16 proteins were up-regulated and 4 proteins were down-regulated in RR-HEp-2 cells. Out of them, 16 radioresistance-related proteins were successfully identified by MALDI-TOF mass spectrometry (Table. 1). The identified proteins could be categorized into six groups by their biological functions: metabolism, transport, cell migration/motility, biosynthesis, chaperone, and anti-apoptosis. The majority of identified proteins took part in metabolism as like enzyme activity, catalysis, mRNA splicing, protein-binding.

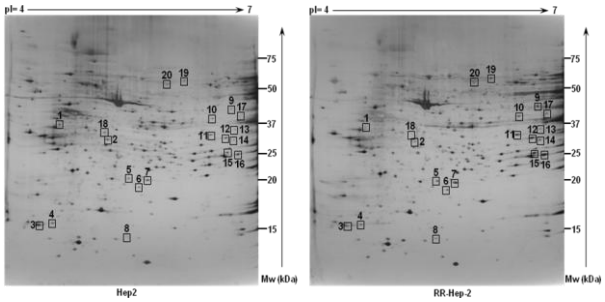

Fig. 4. Comparative two-dimensional gel analysis of differentially expression proteins between parental HEp-2 and radioresistant HEp-2 cells.

No	Name	No	Name
2	RSP1	13	Ribose-phosphate pyrophosphokinase 2
6	Adenine phosphoribosyltransferase	14	Purine nucleoside phosphorylase
7	Peroxiredoxin-2	15	Proteasome subunit type-6
8	RRP1	16	Triosephosphate isomerase
9	RRP2	17	Alcohol dehydrogenase
10	Transaldolase	18	Microtubule-associated protein RP/EB family 1
11	Delta(3,5)-Delta(2,4)-dienoyl-CoA isomerase	19	T-complex protein 1 alpha
12	Proteasome subunit alpha	20	Protein disulfide-isomerase
13	Ribose-phosphate pyrophosphokinase 2		

Table 1. List of identified proteins by MALDI-TOF MS.

3. Conclusions

In this study, we established a unique radioresistant model system utilizing HEp-2 laryngeal cancer cells. Using this cellular model system, 8 radioresistance-related genes and 16 radioresistance-related proteins were identified by applying proteomic and EST data mining methods, respectively. This large scale identification of radioresistance-related molecules using two different methods will provide valuable information for the molecular markers and mechanisms of radioresistance of laryngeal cancer.

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