Genome-Wide cDNA Microarray Screening of Gene Expression Profiles Correlated with Resistance to Anti-Cancer Drug Treatment and Radiation in Oral Squamous Cell Carcinoma

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Summary. Chemotherapy and radiation therapy are an acceptable treatment modality for patients with oral squamous cell carcinomas (OSCC). However, the response of carcinoma to radiation and/or chemotherapy varies in each patient. To choose the proper therapy as well as to avoid untoward side effects, a useful method to predicting the effectiveness of radiation and/or chemotherapy for individual patient must be established. To identify genes correlating with sensitivity to anti-cancer drug and radiation in oral cancer treatment, we performed cDNA microarray analysis of OSCC cell lines. In OSCC cell lines, we compared the gene expression profiles with the sensitivities, that were measured by CD-DST method, to five anti-cancer drugs and with the radiation sensitivity assessed by a standard colony formation assay. In this study, we found significant associations between dozens of gene expression levels and resistance to anti-cancer drugs and radiation.

Key word. gene expression profiles, cDNA microarray, anti-cancer drug, radiation, oral squamous cell carcinoma

Introduction

Chemotherapy and radiation therapy play an important role in OSCC treatment. They allow to improve the overall survival rates and to maintain oral morphology and its important function. However, the response of carcinoma to these therapies varies in each patient, and no survival benefit has been observed in non-responders (Andreadis 2003). Difference among patients with respect to the effectiveness of anti-cancer drugs and/or radiation has been associated with variation in gene expression profiles in cancer cells (Golub 1999, Golub 2001).

Using a cDNA microarray analysis, we obtained comprehensive gene-expression profiles of OSCC cell lines. In present study, we
identified the candidates of molecular markers for the potential of predicting sensitivity to anti-cancer drugs and radiation therapy.

**Materials and Methods**

**Evaluation of sensitivity to anti-cancer drugs using CD-DST**

The prepared tumor cell suspension was added to a collagen solution (Collagen Gel Culture Kit, Nitta Gelatin) and allowed to gel at 37°C in a CO2 incubator. We introduced each of five anti-cancer drugs into the wells. After the incubation for 7 days, Neutral red was added and the cells were fixed by 10% neutral formalin buffer and then quantified by an image analysis.

**Clonogenic survival assay for radiation sensitivity**

OSCC cells were plated and, 48 hr later, they were irradiated with various single radiation doses by a linear accelerator (MBR.1520R, Hitachi Medico, Tokyo), and then incubated for approximately 10 days to allow form to colonies. The survival fractions were calculated as a ratio of plating efficiencies in treated and untreated cells.

**cDNA Microarrays**

Applied Biosystems Human Genome Survey Arrays were used to analyze the transcriptional profiles of RNA samples of OSCC cell lines. Array hybridization (two arrays per sample), Chemiluminescence detection, image acquisition and analysis were performed using Applied Biosystems Chemiluminescence Detection Kit and Applied Biosystems 1700 Chemiluminescent Microarray Analyzer following manufacturer's protocol.

**Identification of the genes associated with resistance to anti-cancer drug and radiation**

Gene spring 7 software (Silicon Genetics) was used to extract assay signal and noise ratio values from the microarray images. To estimate correlation between the expression ratio and sensitivity to each drug, we calculated a Pearson correlation coefficient by the following formula: 

\[ r = \frac{\sum (x_i - \overline{x})(y_i - \overline{y})}{\sqrt{\sum (x_i - \overline{x})^2}(y_i - \overline{y})^2} \]

and we selected genes showing significant correlation (P < 0.01).