学 位 論 文 の 要 旨

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学 位 論 文 名	FAP-1 and NF-κB Expressions in Oral Squamous Cell Carcinoma as Potential Markers for Chemo-radio Sensitivity and Prognosis
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論 文 内 容 の 要 旨

INTRODUCTION

Treatment of oral squamous cell carcinoma (OSCC) involves surgical resection, radiotherapy, chemotherapy or a combination of these treatment modalities. Complete surgical resection for OSCC can alter oral functions and the appearance of the mouth despite recent advances in reconstructive techniques. Chemotherapy and radiotherapy may thus play an important role in both organ preservation and quality of life. However, the clinicopathological criteria for employing chemotherapy and radiotherapy without surgery and predicting outcome of patients have not been established.

Induction of apoptosis after chemo-radiotherapy is correlated with tumor response. Fas (APO-1/CD95), a member of the tumor necrosis factor receptor family, is recognized as a major pathway for the induction of apoptosis. Concomitantly, death receptor signals are tightly regulated by anti-apoptotic factors to avoid inappropriate apoptosis. Fas-associated phosphatase-1 (FAP-1), a cytoplasmic tyrosine phosphatase, binds the cytoplasmic tail of Fas and inhibits Fas-dependent apoptosis. Down-regulation of the FAP-1 expression induces enhancement of the Fas-signaling pathway. FAP-1 is widely expressed in normal human tissue and 78% of tumor cells including OSCC. Recently, it has been reported that nuclear factor kappa B (NF-κB) and p53 are involved in the modulation of FAP-1 expression. However, the clinical influence of FAP-1 expression on patients with OSCC has been unclear.

We considered that FAP-1 might serve as a marker of the efficacy of chemotherapy and/or radiotherapy because it has been considered that FAP-1 expression confers chemo-radio resistance to malignant cells. Therefore, we immunohistochemically examined the expression of FAP-1, NF-κB and p53 in biopsy samples of primary OSCC to evaluate the relation between these expressions and clinicopathological factors or clinical outcome.

MATERIALS AND METHODS

Patients

Fifty patients with histopathological diagnosis of OSCC in biopsy specimens were evaluated. The subjects were patients, who refused surgical treatment, treated by chemotherapy and/or radiotherapy at our department from 1990 to 2002. Of the subjects, 35 were male and 15 female, and mean age was 68.5 years (from 43 to 89 years). In the subjects, patients who died within six months were excluded from this study.

Immunohistochemistry and evaluation of the immunohistochemical stainings

All biopsy samples were fixed with 10% neutral buffered formalin and embedded in paraffin. Three-μm-thick sections of formalin-fixed, paraffin-embedded biopsy samples were processed by streptavidin-biotin-peroxidase complex method using primary antibodies against FAP-1, NF-κB p65, or p53. They were then incubated with streptavidin peroxidase reagents, visualized with diaminobenzidine solution, resulting in a brown precipitate, and counterstained with Mayer's hematoxylin. The immunohistochemical stainings for FAP-1 and NF-κB p65 were analyzed semi-quantitatively using the Remmele score, which took into account both the percentage of positive cells and the intensity of staining. The immunohistochemical staining of FAP-1 was conducted in the cytoplasm. The immunohistochemical staining of NF-κB was conducted in the nucleus and/or cytoplasm using NF-κB p65. The immunohistochemical staining for p53 was also evaluated semi-quantitatively according to the presence or absence of nuclear staining.

Statistical analysis

The Mann-Whitney U-test was used to compare FAP-1, NF-κB or p53 expression with clinicopathological factors. The relationship among FAP-1, NF-κB or p53 expression was examined using Fischer's exact test. Overall survival rates were estimated by the Kaplan-Meier method and compared by a log-rank test. To evaluate the effects of the expressions on the clinicopathological variables and the patients' prognoses, multivariate analysis using the Cox stepwise proportional-hazards model was performed. A hazard ratio of 1 was considered to indicate equivalence for the different levels of a factor. A P-value of less than 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

A summery of the treatment for the 50 patients was as follows. Chemotherapy protocols were based on 5-fluorouracil, platinum, or taxane, combined with or without other anticancer agents. Radiotherapy was carried out as external, intracavitary, or interstitial irradiation, or a combination of these irradiations. The average irradiation dose was 65.1 Gy (range: 38-91 Gy). Over the median follow-up term of 61.2 months (range 7 to 179), 16 of 50 patients died of OSCC and 6 patients died of other diseases. The overall survival rate at 5 years was 60.7%.

Immunoreactivities of FAP-1, NF-κB p65 and p53

FAP-1 was immunohistochemically observed along the cell membrane and/or in the cytoplasm, and its translocation to the cell membrane or submembranous areas supports the theory that FAP-1 is an inhibitor of Fas, while the FAP-1 staining in the cytoplasm may indicate that FAP-1 is stored in the cytoplasmic pool prior to translocation to the submembranous area. In this study, FAP-1-positive reactivity was seen as cytoplasmic staining. FAP-1 immunoreactivity was observed in 26 (52.0%) of the 50 samples.

NF-κB p65 expression was seen in the cytoplasm and/or nucleus. NF-κB, existing as a homodimeric or heterodimeric complex of p50 and p63 subunits, remains inactive in the cytoplasm associated with the NF-κB inhibitory protein (I-κB). Activated NF-κB, released from I-κB, translocates from the cytoplasm into the nucleus and binds to a specific sequence in the promoter of target genes. In this study, NF-κB p65-positive reactivity was

seen as nuclear and/or cytoplasm staining. NF-κB p65 immunoreactivity was observed in 26 (52.0%) of the 50 samples, and cytoplasmic and nuclear staining was observed in 11 samples, only cytoplasmic staining in 13 samples and only nuclear staining in 2 samples.

p53-positive reactivity was seen as nuclear staining. p53 immunoreactivity was observed in 23 (46.0%) of the 50 samples.

FAP-1, NF-κB p65 and p53 expression did not significantly correlate with tumor size, tumor differentiation or clinical stage. Furthermore, there was no significant correlation between sites of tumor and immunoreactivities.

Relationship among the immunoreactivities and survival rate

There was no significant relationship among FAP-1, NF-κB and p53 expression. The relation between FAP-1 expression and prognosis has previously been investigated only in ovarian cancers. In that series, FAP-1 was founded to be expressed in 97.8% of patients, and there was no significant correlation between the intensity of FAP-1 immunohistochemical staining and the response to chemotherapy or the survival rate. In this study, the 24 FAP-1-negative patients showed a significantly better prognosis than the 26 FAP-1-positive patients ($P = 0.0409$).

NF-κB overexpression was associated with poor prognosis and resistance to chemoradiotherapy in head and neck carcinoma. In this study, the 24 NF-κB-negative patients showed a significantly better prognosis than the 26 NF-κB -positive patients ($P = 0.0018$).

In our comparison of the survival rates between the p53-positive and -negative subjects, no significant difference was found. Previous reports examining the correlation between p53 status and the clinical outcome for patients with head and neck squamous cell carcinoma, including OSCC, have been inconclusive. These unclear results might be due to factors such as small or heterogeneous subjects populations, or differences in the pretreatment of the tissue, antibodies applied, cut off points for the percentage or intensity of stained cells to be considered as positive, and general conditions of the staining.

Furthermore, 16 patients with coexpression of FAP-1 and NF-κB showed a significant poorer prognosis than 34 patients with expression of neither or only one of these makers ($P = 0.0011$). Multivariate analysis using Cox's proportional hazards model showed that FAP-1 expression, NF-κB expression, clinical stage and age were significant independent variables for survival (clinical stage: P=0.0016; age: P=0.0016; FAP-1: P=0.0366; NF-κB: P=0.0314).

CONCLUSION

FAP-1 expression was not correlated with NF-κB or p53 expression in this immunohistochemical study, despite the fact that previous studies have suggested FAP-1 expression might be controlled by NF-κB and p53. And these expressions were not correlated with clinicopathological parameters. It is likely that regulation of FAP-1 by NF-κB and p53 is more complex than previously thought. FAP-1 expression and NF-κB expression were revealed to be related to chemo-radio sensitivity and to be feasible as prognostic factors in OSCC. Clinically, chemo-radio sensitivity predicted from the expressions would be an influential factor to select of treatment modalities. Further understanding of the involvement of FAP-1, NF-κB and p53 in the induction of apoptosis could contribute to the development of effective strategies for chemo-radio resistance.