学 位 論 文 の 要 旨

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学 位 論 文 名	Is Nucleus Accumbens-Associated Protein 1 A Feasible Marker for Distinguishing Oral Malignancies from Non-malignancies? First Investigation of Nucleus Accumbens-Associated Protein 1 Expression in Oral Lesions
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論 文 内 容 の 要 旨

INTRODUCTION

Nucleus accumbens-associated protein 1 (NAC1) is a member of the Pox virus and Zinc finger/Bric-a-brac Tramtrack Broad complex family of proteins that mediates several cellular functions including proliferation, apoptosis, transcription control, and cell morphology maintenance. We evaluated NAC1 expression in normal oral epithelium (NOE) and various oral lesions to verify whether NAC1 is a feasible marker for distinguishing oral malignancies from non-malignancies. This preliminary study is the first study on NAC1 expression in oral lesions.

MATERIALS AND METHODS

Subjects comprised 165 patients (88 men, 77 women; mean age, 65.2 years), including 32 with lichen planus (LP), 19 with hyperkeratosis (HK), 67 with epithelial dysplasia (ED), 10 with carcinoma in situ (CIS), and 37 with oral squamous cell carcinoma (OSCC). NOE was taken from 15 healthy participants (7 men, 8 women; mean age, 61.9 years).

Biopsy specimens (formalin-fixed paraffin-embedded sections) were treated with NAC1 mouse monoclonal antibody (diluted 1:1,000 overnight at 4°C) after deparaffinization. Sections were then incubated in a substrate solution consisting of 0.05% diaminobenzidine tetrahydrochloride.

Under a standard light microscope, images were captured with an attached digital camera to estimate the number of NAC1-positive cells. NAC1 immunoreactivity intensity was then evaluated in Image J v1.47 (National Institute of Health, Bethesda, MD) by analyzing the brightness of each pixel in RGB images. In cases of OSCC, primary sites and pathologic N classification (pN) were examined.

The results were analyzed using R. app GUI 1.64 for Mac OS (R Foundation for Statistical Computing, Vienna, Austria). NAC1 LIs and the immunoreactivity intensity were individually compared between the normal and other lesions using the Kruskal-Wallis test or ANOVA. Statistical analysis using ANOVA or Kruskal-Wallis test was indicated following Bartlett's test.

In cases of OSCC, statistical differences between primary sites as well as lymph node metastases (pN, number of metastatic lymph nodes and level of involvement) and NAC1 LIs / NAC1 immunoreactivity intensity were determined using the ANOVA for continuous variables. A p value ≤ 0.001 was considered significant.

The study protocol was approved by the Ethics Committee of Shimane University Hospital and written informed consent was obtained from all subjects.

RESULTS AND DISCUSSION

In NOE and CIS, NAC1-positive cells were strongly expressed in the basal cell layers, and uniformly distributed in all epithelial layers. In ED, HK and LP, NAC1-positive cells were distributed mainly from the basal cell to spinous layers, and were also found in the proliferating area of oral squamous cell carcinoma .

NAC1 labeling indices correlated strongly with NAC1 immunoreactivity intensity. NAC1 expression was observed not only in NOE, but also in oral premalignancies and malignancies. Significant differences among NOE and each lesion ($p<0.001$, Kruskal-Wallis test) was seen in the NAC1 LIs. Significant differences were observed among upon detailed grading of oral ED by WHO classification and the differentiation of OSCC, and other lesions including NOE ($p<0.001$, ANOVA).

Significant differences were seen in the NAC1 LIs among mild, moderate, and severe dysplasia ($p<0.001$, ANOVA). However, no significant differences were seen between each histological type of NAC1 LIs from the viewpoint of squamous cell carcinoma differentiation ($p=0.91$, ANOVA).

The pixel count was 119.6 ± 10.7 for NOE, 119.2 ± 7.3 for OSCC, 132.5 ± 9.1 for ED, 124.1 ± 9.7 for LP, and 138.8 ± 4.9 for HK. Significant differences were seen among each type of lesion, including NOE in the NAC1 immunoreactivity intensity ($p<0.001$, ANOVA).

Significant differences were seen among mild, moderate, and severe dysplasia in the pixel count ($p<0.001$, ANOVA). However, regarding the pixel count from the viewpoint of squamous cell carcinoma differentiation, no significant differences were seen between each histological type ($p=0.48$, ANOVA).

No significant differences were seen in the NAC1 LIs (p=0.73, ANOVA) and immunoreactivity intensity (p=0.24, ANOVA) between the primary sites and cervical lymph node involvement.

In this study, NAC1 expression was stronger in malignant tissues including CIS, which can be expected since OSCC has a high potential for both invasion and cervical lymph nodes metastasis. These findings are reasonable, as NAC1 was reported to be overexpressed in cervical squamous cell carcinoma, adenocarcinomas and serous ovarian carcinoma. Some clinical investigations have reported the relationship between overexpression of NAC1 and the clinical behavior of malignancies and patient prognosis. However, this study showed no significant associations between NAC1 expression and lymph node involvement in OSCC. As the reason for these results is unclear, further study is needed to elucidate the relationship between NAC1 expression and the clinical behavior of OSCC.

This study also showed NAC1 expression in NOE was as high as that in malignant tissue. NAC1 is a primary Nanog-interacting protein that is part of the protein regulatory complex responsible for maintaining pluripotency. NAC1 has been shown to regulate transcription of the transcription factors, Nanog, Oct4 and Sox2, which are essential for the development and maintenance of the pluripotent state of embryonic stem cells. Sox2-Cre-ER; Rosa26-LSL-EYFP mouse model showed that Sox2 is expressed by basal layer stem cells for at least 10 months after labeling in the dorsum of the tongue. When considering the strong expression of NAC1 in NOE, Sox2 was thought to play an important role in downregulating the epithelial cells derived from the ectoderm, while NAC1 likely participated in transcriptional regulation of Sox2 in the maintenance of cell pluripotency.

CONCLUSION

Though there found difference in NAC1 expression in various oral lesions, NAC1 is not a definitive marker for distinguishing oral malignancies from non-malignancies.