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.

論文審査及び最終試験又は学力の確認の結果の要旨

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【論文審査の結果の要旨】

口腔・咽頭がんの年間死亡者数は約7,000人を超えており、所属リンパ節への転移の有無や予後の予測、および良悪性の鑑別に有用なマーカーを見出すことが必要である。近年、婦人科領域や膵癌などで、転写制御因子であるNucleus Accumbens-Associated Protein 1 (NAC1) が、予後や薬剤耐性に関連するマーカーとして注目されているが、口腔領域ではそのような報告はない。申請者らは、口腔扁平上皮とそれに由来する病変におけるNAC1の発現を明らかにし、悪性腫瘍におけるリンパ節転移の有無との関係や良悪性の判定を明らかにすることを目的に以下の研究を行った。

材料として1980年から2013年までに、島根大学医学部歯科口腔外科にて採取された口腔病変の生検検体180例（正常扁平上皮15例、扁平苔癬32例、過角化症19例、上皮異形成症67例、上皮内癌10例、扁平上皮癌37例）を用いた。これらに対してNAC1の免疫染色を行い、その標識率を算出し、画像解析ソフトImage Jで染色強度を数値化した。それぞれのデータを統計解析により群間比較を行い、扁平上皮癌に関しては原発部位や分化度、およびリンパ節転移との関連を検討した。

結果は正常扁平上皮、扁平苔癬、過角化症、上皮異形成症、扁平上皮癌では５群間でNAC1標識率、染色強度ともに有意差($p<0.001$)を示した。また、正常扁平上皮、上皮異形成の軽度、中等度、高度、上皮内癌、扁平上皮癌の高分化、中分化、低分化型の８群間でもNAC1標識率、NAC1染色強度ともに有意差($p<0.001$)を示した。ただし、扁平上皮癌について分化型、原発部位、リンパ節転移数、転移レベルなどとの相関を解析したが有意差はなかった。しかしながら、上皮異形成症、上皮内癌、扁平上皮癌のみをχ^2検定にて解析したところ、カットオフ値を標識率50%、染色強度124ピクセルに設定することにより、異形成と癌の鑑別にNAC1の免疫染色が有用である可能性が示唆された。

本研究結果からはNAC1の発現と、近年考えられている上皮内癌のタイプとの関連が不明である点や、正常上皮と癌の染色態度が類似していた点など、今後さらに検討すべき課題を残しているが、病理診断上非常に興味ある結果といえる。

【最終試験又は学力の確認の結果の要旨】

申請者らは免疫染色を用いて、NAC1タンパクの発現の程度が、口腔粘膜上皮異形成と癌との鑑別に有用である可能性を示した。今後さらなる検討が必要であるが、口腔粘膜病変の診断を行う上で興味ある結果であり、関連知識も豊富であることから学位授与に値すると判断した。（主査：丸山理留敬）

申請者は、口腔領域の種々の疾患におけるNAC1蛋白の発現を免疫組織学的手法にて検討し、更に、発現パターンと疾患の鑑別の可能性について示した。本研究は、口腔領域疾患の病態解明に寄与するものであり、学位授与に値すると判断した。（副査：熊倉俊一）

申請者らは、NAC1タンパクの発現の程度が、口腔粘膜の上皮異形成症と癌との鑑別に有用である可能性を示した。今後スクリーニングでの感度特異度などの検討が必要であるが、臨床上の応用が期待される研究成果である。公開審査では的確に質疑応答し、関連知識も豊富であることから学位授与に値すると判断した。（副査：小林裕太）