Int. J. Oral Maxillofac. Surg. 2019; 48: 1265–1272
https://doi.org/10.1016/j.ijom.2019.02.012, available online at https://www.sciencedirect.com

International Journal of Oral & Maxillofacial Surgery

Clinical Paper
Head and Neck Oncology

Nuclear protein of the testis midline carcinoma in the oral cavity: retrospective review of those initially diagnosed as poorly differentiated squamous cell carcinoma using an anti-C52B1 antibody

H. Aizawa[1,2], S. Yamada[2], H. Sakai[2], H. Otakiri[2], D. Akita[2], T. Gibo[2], H. Kurita[2]

[1]Department of Dentistry and Oral Surgery, Okaya Municipal Hospital, Okaya, Japan; [2]Department of Dentistry and Oral Surgery, Shinshu University School of Medicine, Matsumoto, Japan

H. Aizawa, S. Yamada, H. Sakai, H. Otakiri, D. Akita, T. Gibo, H. Kurita: Nuclear protein of the testis midline carcinoma in the oral cavity: retrospective review of those initially diagnosed as poorly differentiated squamous cell carcinoma using an anti-C52B1 antibody. Int. J. Oral Maxillofac. Surg. 2019; 48: 1265–1272.

Abstract. Nuclear protein of the testis (NUT) midline carcinomas (NMC) are malignant epithelial tumours that have chromosomal rearrangements of the gene encoding NUT at 15q14. NMC is typically an aggressive fatal cancer, clinically overlaps with other carcinomas, and differential diagnosis is difficult. The purpose of this study was to investigate NMC in poorly differentiated oral squamous cell carcinoma (OSCC) with a retrospective analysis based on anti-C52B1 immunohistochemical staining. An anti-C52B1 antibody was used for immunohistochemical staining in all 27 primary tumours, and the prevalence and pathological features of NMC in the oral cavity were examined. Only two of 27 cases (7.4%) were C52B1 immunopositive. Both positive patients were women aged 38 and 43 years — younger than the other C52B1-negative patients, whose average age was 65.6 years (range 41–83). The primary sites were the right side of the floor of the mouth and the left side of the tongue. They had a poor prognosis and died within 8 months postoperation compared with the median overall survival time of 60.2 months for patients with other poorly differentiated squamous cell carcinoma. The pathological findings of their primary tumours were similar to typical poorly differentiated OSCC.

Key words: NUT midline carcinoma; anti-C52B1 antibody; poorly differentiated; oral squamous cell carcinoma.

Accepted for publication 20 February 2019
Available online 12 March 2019

0901-5027/01001265 + 08

Nuclear protein of the testis (NUT) midline carcinoma (NMC) is a malignant epithelial tumour caused by mutation of the NUT gene (BRD4-fusion) on chromosome 15q14 of normal cells. NMC arises in epithelial tissue on the midline of the body including the trachea, thymus, mediastinum, and bladder. NMC has aggressive malignancy with high mortality and poor prognosis and arises preferentially in young people of both sexes[1,2]. There were 49 cases of NMC reported in the head and neck region according to the International NMC Registry[3–7], but only two cases in the Japanese population[7]. Because the clinical features of NMC resemble those of poorly differentiated oral squamous cell carcinoma (OSCC), differential diagnosis is difficult[1,2]. However, immunohistochemical staining with anti-C52B1 antibody is useful to diagnose NMC with high sensitivity[8].

The reported sites of NMC were in the nasal cavity, nasopharynx in three, oropharynx in one, hypopharynx in one, larynx in one, salivary gland in two, unknown in five, and none in the oral cavity. Therefore, the purpose of this study was to investigate NMC in poorly differentiated oral squamous cell carcinoma with a retrospective analysis based on anti-C52B1 immunohistochemical staining.

Materials and methods

The study protocol was approved by the Ethics Committee of Shinshu University School of Medicine (No. 3231). The medical records of all patients treated at the Department of Oral and Maxillofacial Surgery, Shinshu University Hospital for poorly differentiated OSCC treated between April 2003 and May 2015 were retrospectively reviewed and investigated.

To diagnose NMC among poorly differentiated OSCC, immunohistochemical staining of surgical specimens was performed with anti-C52B1 antibody. Serial 4-μm-thick sections were sliced from tissue blocks. Sections were deparaffinized in xylene, soaked in target retrieval solution buffer (Dako, Glostrup, Denmark), and placed in an autoclave at 121 °C for 5 min for antigen retrieval. Endogenous peroxidase was blocked by incubating sections with 0.3% H_2O_2 in methanol for 30 min. Immunohistochemical staining was performed using the Envision system (Envision+, Dako, Carpinteria, CA). The primary antibody was anti-C52B1 (#3625, Cell Signaling Technology, Danvers, MA, USA, dilution 1:50). Sections were incubated with the primary antibody overnight at 4 °C. Reaction products were visualized by immersing the sections in diaminobenzidine (DAB) solution, and the samples were counterstained with Meyer's hematoxylin and mounted. Negative controls were prepared by replacing the primary antibody with phosphate-buffered saline. The immunoreactivity of C52B1 was scored based on immunoreactive cells over 50% positive[9].

For statistical analysis, a Kaplan–Meier method was used to examine overall survival curves. Statistical analyses were performed using StatMate IV (Atms Co., Tokyo, Japan).

Results

Twenty-seven patients (14 males and 13 females) who were diagnosed and treated for poorly differentiated OSCC were included (Table 1). The median age was 66 years (range: 38–84 years). The most frequent site was the tongue in 10 cases (37.0%), lower gingiva in five (18.5%), upper gingiva in four (14.8%), and buccal mucosa in three (11.1%), floor of mouth in three (11.1%), and palate in two (7.4%). The follow-up period was 31.9 ± 17.1 months. The 5-year overall survival rate of poorly differentiated OSCC was 37.2%. Immunoreactivity of C52B1 was detected in the nuclei of tumour cells. Among 27 cases, NMC was diagnosed in two cases (7.4%). A Kaplan–Meyer curve of overall survival between NMC and other poorly differentiated OSCC is shown in Fig. 1. NMC had a poor prognosis, and patients died within 8 months postoperation compared with the median overall survival time of 60.2 months for patients with other poorly differentiated squamous cell carcinoma.

Case 1 (No. 26)

A 38-year-old female was referred to our hospital with progression of ulceration on the right side of the tongue in April 2014. There were no past medical or family histories. Physical examination revealed a poorly defined 42×15 mm mass with induration and ulceration in the right side of the oral floor to the lower surface of the tongue (Fig. 2A). The right submandibular, upper cervical, and peri-clavicular lymph nodes were palpable with tenderness. Enhanced T1-weighted magnetic resonance imaging (MRI) revealed a $35 \times 32 \times 16$ mm, heterogeneous, moderate enhanced mass lesion with poorly defined margins in the right side of the oral floor to the lower surface of the tongue (Fig. 3A). Enhanced T2-weighted MRI also revealed the same mass lesion with high-signal enhanced margins (Fig. 3B). Positron emission tomography-computed tomography (PET-CT) revealed an accumulation of F-18 deoxyglucose (FDG) in the right submandibular, upper cervical, and peri-clavicular lymph nodes (Fig. 3C). No metastases to the lung or liver were detected by CT and PET-CT examinations. Laboratory data were normal.

A biopsy was performed, and a pathological diagnosis of poorly differentiated OSCC was obtained. The clinical diagnosis of oral floor carcinoma (cT3N2bM0, stage IVA) was made according to the American Joint Committee on Cancer system 7th edition[10]. Induction chemotherapy with nedaplatin (CDGP) 40 mg/m^2 and docetaxel (TXT) 30 mg/m^2 was administered through one cycle, and treatment resulted in a partial response with 61.3% reduction, according to RECIST guideline (version1.1)[11]. A wide local resection concomitant with right radical neck dissection (levels I–V), left supra-omohyoid neck dissection (levels I–III), and reconstruction with anterolateral thigh flap were performed under general anaesthesia. The pathological findings of the surgical specimen revealed an invasive proliferation of atypical epithelial cells with eosinophil cytoplasm under the oral floor mucosa (Fig. 2B). Tumour cells had diffuse invasion and growth without cancer nests and cancer pearls. The pathological diagnosis of the surgical specimen was also poorly differentiated squamous cell carcinoma and the surgical margin was free of the tumour. Among 29 extirpated lymph nodes from right side and 18 from left side, 18 lymph node metastases were in the right levels IB, IIA, III, IV and V. An extracapsular spread (ECS) was detected in the right level III lymph node metastasis. Because of the diagnosis of pT2N2bM0, stage IVA (UICC, 7th), postoperative concurrent chemoradiotherapy (CCRT) with high-dose cisplatin (CDDP) was administered (total 63 Gy and CDDP 100 mg/m^2, triweekly) and finished. After discharge, seven courses of chemotherapy with CDGP 40 mg/m^2 and TXT 30 mg/m^2 were administered at an outpatient clinic. Although there was no recurrence or metastasis of the tumour during the postoperative follow-up period, the patient died of an unknown cause 7 months postoperation. However, since the bereaved family did not accept the pathological autopsy, it is impossible to specify the precise cause of death.

Immunohistochemical analysis with anti-C52B1 antibody revealed characteristic intranuclear spot-like positivity in parts of

Table 1. Clinical characteristics between nuclear protein of the testis midline carcinoma (NMC) and poorly differentiated squamous cell carcinoma.

Case number	Age	Sex	C52B1 immunoreaction	Location	TNM classification (7th edt.)	Number of cervical lymph node metastases	Treatment	Prognosis (months)
1	63	Male	Negative	Lower Gingiva	T1N0M0, Stage I	0	Surgery	Alive (8.1)
2	66	Male	Negative	Buccal mucosa	T4aN0M0, Stage IVA	0	Surgery + radiotherapy + chemotherapy	Death (60.1)
3	77	Female	Negative	Lower Gingiva	T2N1M0, Stage III	1	Surgery + radiotherapy + chemotherapy	Death (16.6)
4	82	Female	Negative	Palate	T2N0M0, Stage II	0	Surgery	Alive (63.3)
5	64	Female	Negative	Upper Gingiva	T1N1M0, Stage III	1	Surgery + radiotherapy + chemotherapy	Death (33.1)
6	54	Female	Negative	Tongue	T2N0M0, Stage II	0	Surgery	Alive (63.3)
7	68	Female	Negative	Tongue	T1N0M0, Stage I	0	Surgery	Alive (28.5)
8	81	Female	Negative	Tongue	T1N0M0, Stage I	0	Surgery	Death (47.6)
9	77	Male	Negative	Tongue	T2N0M0, Stage II	0	Surgery + radiotherapy	Alive (35.5)
10	75	Male	Negative	Palate	T4aN0M0, Stage IVA	0	Surgery + radiotherapy + chemotherapy	Death (45.0)
11	69	Male	Negative	Floor of Mouth	T2N0M0, Stage II	0	Surgery + chemotherapy	Alive (63.3)
12	62	Male	Negative	Tongue	T1N0M0, Stage I	0	Surgery	Alive (41.6)
13	83	Female	Negative	Upper Gingiva	T2N0M0, Stage II	0	Surgery	Alive (46.1)
14	65	Male	Negative	Floor of Mouth	T2N0M0, Stage II	0	Surgery	Alive (35.9)
15	77	Male	Negative	Lower Gingiva	T2N2bM0, Stage IVA	3	Surgery + radiotherapy	Alive (29.0)
16	66	Male	Negative	Upper Gingiva	T2N0M0, Stage II	0	Surgery + radiotherapy + chemotherapy	Death (17.0)
17	84	Female	Negative	Buccal mucosa	T2N0M0, Stage II	0	Surgery	Alive (27.4)
18	74	Male	Negative	Tongue	T2N1M0, Stage III	1	Surgery	Death (21.3)
19	61	Male	Negative	Buccal mucosa	T2N0M0, Stage II	0	Surgery	Alive (24.0)
20	50	Male	Negative	Tongue	T1N0M0, Stage I	0	Surgery + radiotherapy + chemotherapy	Alive (17.0)
21	41	Male	Negative	Tongue	T2N0M0, Stage II	0	Surgery + radiotherapy	Alive (32.3)
22	47	Female	Negative	Tongue	T4aN1M0, Stage IVA	1	Surgery + radiotherapy + chemotherapy	Alive (15.2)
23	61	Male	Negative	Upper Gingiva	T4aN0M0, Stage IVA	0	Surgery + radiotherapy	Alive (19.3)
24	75	Female	Negative	Lower Gingiva	T4aN0M0, Stage IVA	0	Surgery + radiotherapy + chemotherapy	Alive (18.1)
25	44	Female	Negative	Lower Gingiva	T4aN2bM0, Stage IVA	3	Surgery + radiotherapy + chemotherapy	Alive (32.3)
26	38	Female	Positive	Floor of Mouth	T3N2bM0, Stage IVA	17	Surgery + radiotherapy + chemotherapy	Death (8.8)
27	43	Female	Positive	Tongue	T2N2cM0, Stage IVA	5	Surgery + radiotherapy + chemotherapy	Death (10.3)

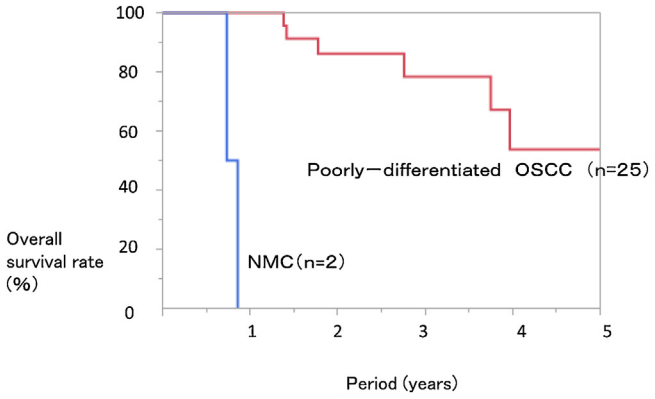

Fig. 1. Kaplan–Meyer curves of overall survival in nuclear protein of the testis midline carcinoma (NMC) and poorly differentiated oral squamous cell carcinoma (OSCC). NMC had a poor prognosis and patients died within 8 months postoperation compared with the median overall survival time of 60.2 months for other poorly differentiated squamous cell carcinomas.

the growing tumour cells (Fig. 2C). Because the positive rate of anti-C52B1 antibody was over 50% of the tumour cells, a diagnosis of NMC was confirmed retrospectively. The immunoreactive profile with anti-Ki67 antibody was consistent with that of C52B1 (Fig. 2D), and immunoreactivity for p53 was negative.

Case 2 (No. 27)

A 43-year-old female was admitted to a local hospital with swelling and pain on the left side of the tongue in April 2013. She was diagnosed with poorly differentiated tongue squamous cell carcinoma (cT2N2cM0, stage IVA, UICC 7th) and induction chemotherapy was administered with docetaxel (DOC) 60 mg/m^2 and CDGP 100 mg/m^2 at the hospital. The treatment had no effect. The patient was referred to our hospital for surgery. There were no past medical or family histories. Physical examination revealed a poorly defined 30 × 22 mm mass with induration with ulceration on the left side of the tongue (Fig. 4A). The right submental, submandibular, and upper cervical lymph nodes and left submental lymph nodes were palpable. Enhanced T1-weighted MRI revealed a 31 × 26 × 16 mm, heterogeneous, moderate enhanced mass lesion with poorly defined margins on the left side of the tongue (Fig. 5A). Enhanced T2-weighted MRI also showed the same mass lesion with high-signal enhanced margins (Fig. 5B). PET-CT revealed an accumulation of FDG in the right submental, submandibular, and upper cervical lymph nodes and left submental lymph nodes with suspicious lymph node metastasis (Fig. 5C). No metastases to the lung or liver were detected by CT and PET-CT examinations. Laboratory data were normal. A wide local resection concomitant

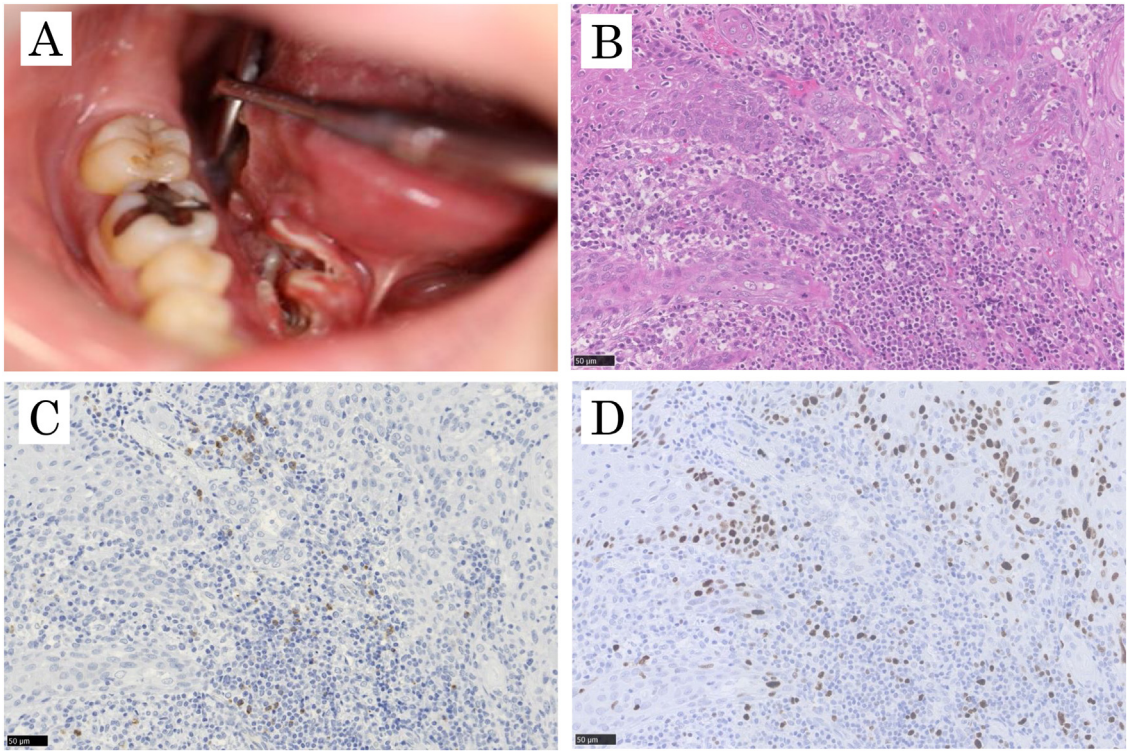

Fig. 2. Nuclear protein of the testis midline carcinoma (NMC): right side of the oral floor to the tongue. (A) Physical examination revealed a poorly defined 42 × 15 mm mass with induration and ulceration in the right side of the oral floor to the lower surface of the tongue. (B) Hematoxylin and eosin (H&E) staining of the surgical specimen revealed an invasive proliferation of atypical epithelial cells with eosinophil cytoplasm under the oral floor mucosa. Tumour cells had diffuse invasion and growth without cancer nests and cancer pearls. The pathological diagnosis of the surgical specimen was also poorly differentiated squamous cell carcinoma. (C) Immunohistochemical analysis with anti-C52B1 antibody revealed characteristic intranuclear spot-like positivity in parts of the growing tumour cells. Because the positive rate of the anti-C52B1 antibody was over 50% of the tumour cells, a diagnosis of NMC was confirmed retrospectively. (D) Immunohistochemical analysis with anti-Ki67 antibody. Magnification (B–D): 400 × .

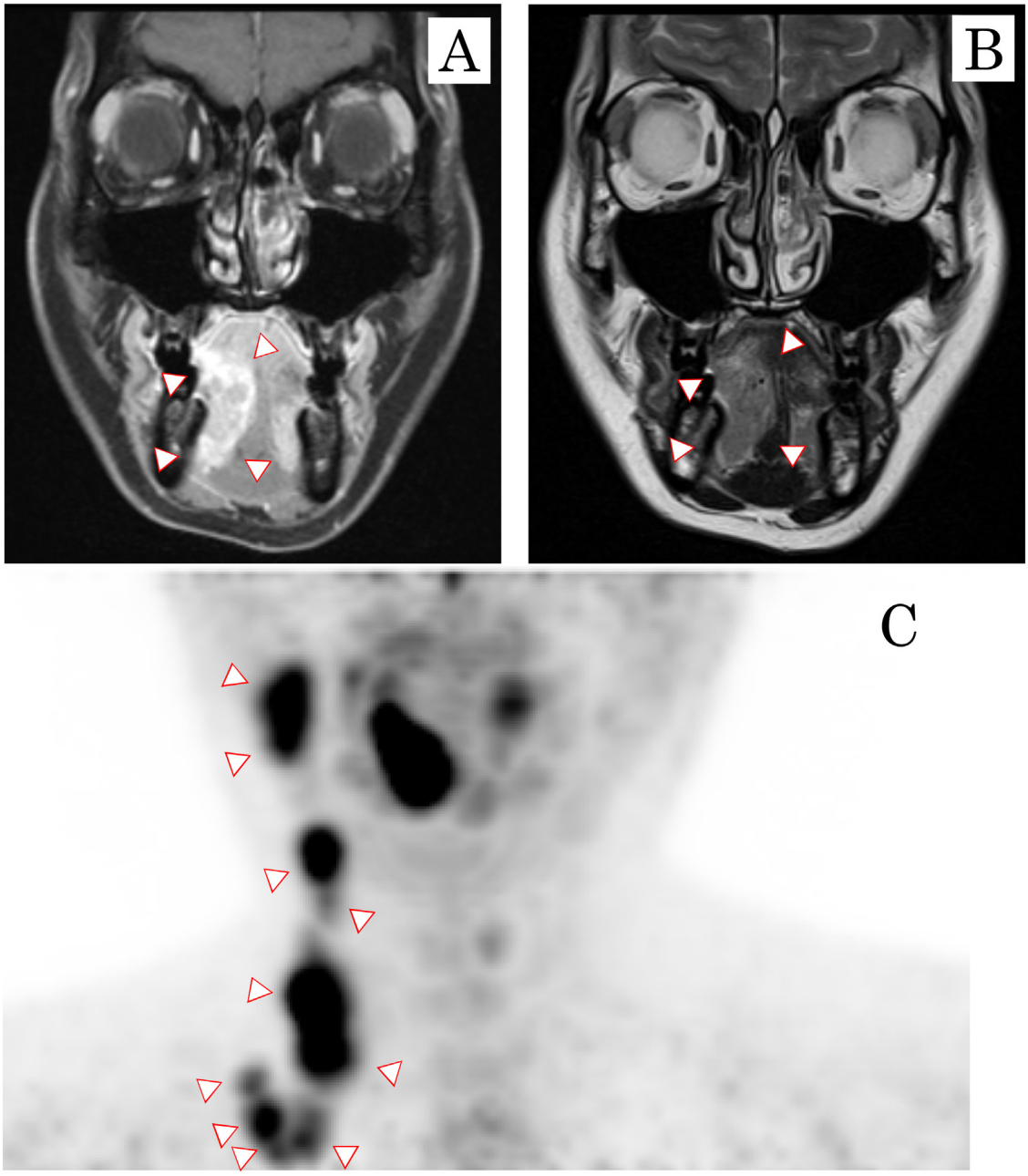

Fig. 3. (A) Enhanced T1-weighted magnetic resonance imaging (MRI) revealed a 35 × 32 × 16 mm, heterogeneous, moderate enhanced mass lesion with poorly defined margins in the right side of the oral floor to the lower surface of the tongue. (B) Enhanced T2-weighted MRI also revealed the same mass lesion with high-signal enhanced margins. (C) Positron emission tomography-computed tomography (PET-CT) revealed an accumulation of F-18 deoxyglucose (FDG) in the right submandibular, upper cervical, and peri-clavicular lymph nodes.

with bilateral radical neck dissection (levels I–V) and reconstruction with anterolateral thigh flap were performed under general anaesthesia. The pathological findings of the surgical specimen revealed an infiltrating proliferation of atypical epithelial cells with eosinophilic cytoplasm under the submucosa. Atypical cells did not make obvious alveolar lesions, but diffuse infiltrating and proliferating of atypical cells were observed. No keratinized pearl formation was observed.

Tumour cells were squamous epithelial-like cells with almost no tendency to keratinize, and irregular nuclei with clear nucleoli were observed (Fig. 4B). A pathological diagnosis of surgical specimens was also poorly differentiated squamous cell carcinoma, and the surgical margin was free of the tumour. Among 25 extirpated lymph nodes from right side and 23 from left side, lymph node metastases were observed 1 in the right levels IA, 2 in the right IIA, 1 in the left levels IA, and 1 in the left IIA. ECS was detected in the left level III lymph node metastasis. Because of the diagnosis of pT2N2cM0, stage IVA, postoperative CCRT with CDDP 90 mg/m^2 and 5-Fluorouracil (5-FU) 900 mg/m^2) was administered. However, CCRT was suspended because of severe oral mucositis and dermatitis at

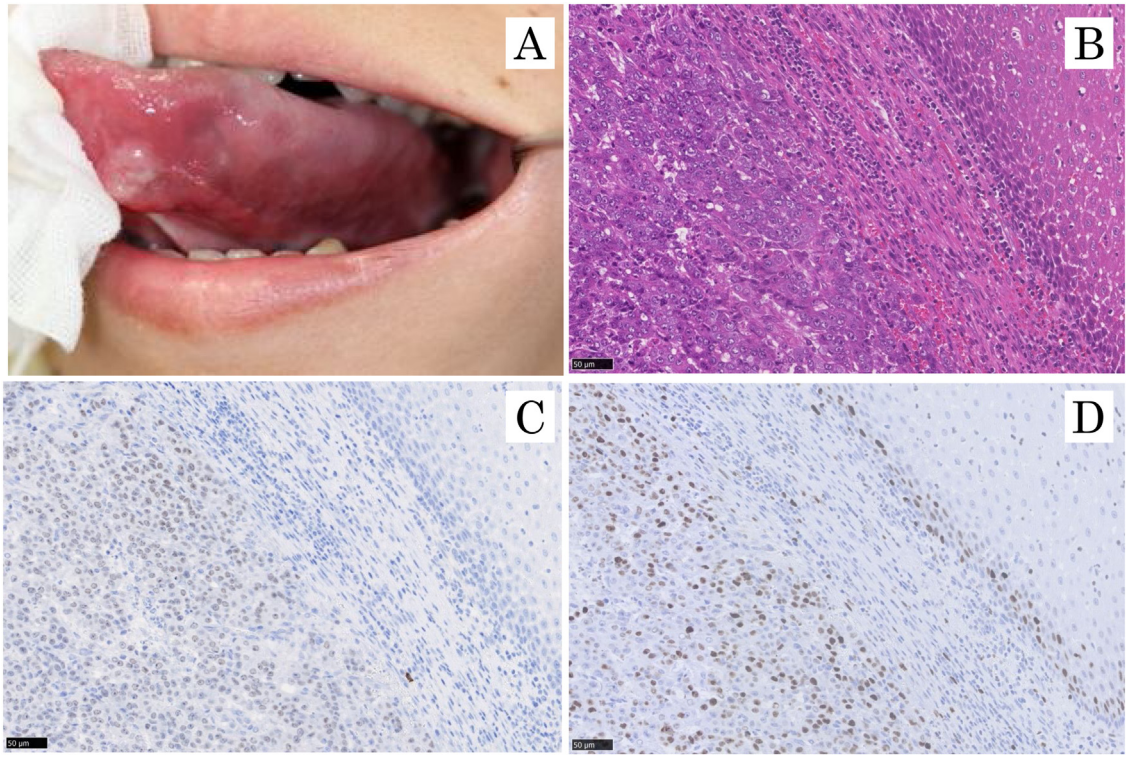

Fig. 4. Nuclear protein of the testis midline carcinoma (NMC): left side of the tongue. (A) Physical examination revealed a poorly defined 30 × 22 mm mass with induration and ulceration on the left side of the tongue. (B) Hematoxylin and eosin (H&E) staining. Tumour cells were squamous epithelial-like cells with almost no tendency to keratinize, and irregular nuclei with clear nucleoli were observed. The pathological diagnosis of the surgical specimen was also poorly differentiated squamous cell carcinoma. (C) Immunohistochemical analysis with anti-C52B1 antibody revealed characteristic intranuclear spot-like positivity in parts of the growing tumour cells. Because the positive rate of the anti-C52B1 antibody was over 50% of the tumour cells, a diagnosis of NMC was confirmed retrospectively. (D) Immunohistochemical analysis with anti-Ki67 antibody. Magnification (B–D): 400×.

16.2 Gy. Lung metastasis was detected 6 months postoperation. The lung metastasis lesion grew rapidly, and the patient died of respiratory failure caused by the lung metastasis 8 months postoperation.

Immunohistochemical analysis with anti-C52B1 antibody revealed characteristic intranuclear spot-like positivity in parts of the growing tumour cells (Fig. 4C). Because the positive rate of anti-C52B1 antibody was over 50% of the tumour cells, a diagnosis of NMC was confirmed retrospectively. An immunoreactive profile with anti-Ki67 antibody was partially positive consistent with that of C52B1 (Fig. 4D), and immunoreactivity for p53 was negative.

Discussion

NMC was first defined in 2004, and this malignant epithelial tumour is caused by mutation of the NUT gene such as a BRD-4 fusion[1–4,12]. Because this disease has only been recognized recently, misdiagnoses, such as undifferentiated carcinoma, poorly differentiated squamous cell carcinoma, Ewing sarcoma, sinonasal undifferentiated carcinoma, thymic carcinoma and neuroblastoma, are common[13]. Kubonishi et al. reported the first case of NMC in a 22-year-old female diagnosed with thymic carcinoma; chemoradiotherapy was administered, but she died from tumour growth 106 days after the first visit[14,15]. Because NMC has high proliferative potential, there is a poor prognosis, a high mortality rate, and an average survival period of less than 1 year[16]. The median overall survival time is 6–7 months[4]. Multiple cervical lymph node metastases at several levels and ECS were detected in both cases. In case 2, postoperative CCRT was suspended, which might affect the prognosis. For the two cases of NMC in this study, one patient died of an unknown cause, and the other died of lung metastasis 8 months after surgery. These cases might suggest that NMC in the oral cavity has a possibility of high mortality. Although postoperative radiation therapy is an important prognostic factor for NMC[4], the standard treatment for NMC is uncertain. The establishment of the treatment strategy for NMC is needed based on large number of NMC case series.

Because NMC is a relatively rare malignancy recognized only recently, it is difficult to distinguish morphologically by histological analysis from other poorly differentiated carcinomas, and the incidence of NMC is unknown. Among poorly differentiated carcinomas arising in non-smokers' upper airways and upper digestive tracts, the incidence of NMC was 7–20%[14,17]. Although NMC is more frequent in young people, there is no difference in the frequency between males and females and NMC occurs in every age group[17]. Based on a small number of cases, the incidence of NMC in this study was 7%, which was consistent with a previous report[18]. It is difficult to diagnose NMC because chromosome staining by fluorescent in situ hybridization (FISH) or detection of amplification of the BRD4-NUT gene by real-time polymerase chain reaction is needed. However, an anti-C52B1 monoclonal antibody specific for NUT was developed with a sensitivity of 87% and specificity of 100% compared with FISH[8].

Histologically, the NMC was a solid or sheet-shaped proliferation composed of small and undifferentiated tumour cells with a large N/C ratio or keratinization,

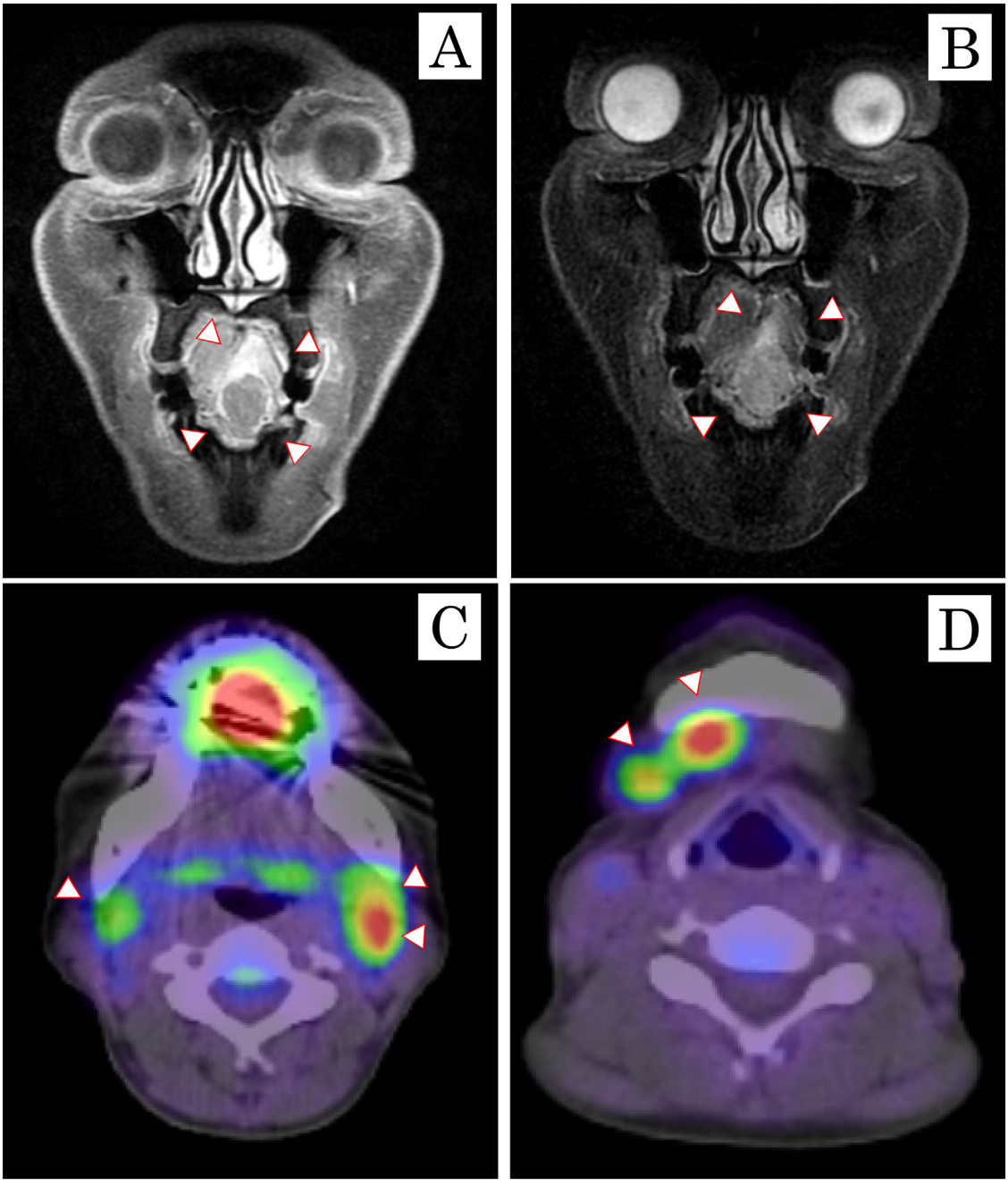

Fig. 5. (A) Enhanced T1-weighted magnetic resonance imaging (MRI) revealed a 31 × 26 × 16 mm, heterogeneous, moderate enhanced mass lesion with poorly defined margins on the left side of the tongue. (B) Enhanced T2-weighted MRI also revealed the same mass lesion with high-signal enhanced margins. (C) Positron emission tomography-computed tomography (PET-CT) revealed an accumulation of F-18 deoxyglucose in the right submental, submandibular and upper cervical lymph nodes and left submental lymph nodes with suspicious lymph node metastasis.

but NMC is difficult to definitively diagnose because of the lack of morphological characteristics[17–20]. Neutrophil invasion also occurs[9,17–20]. Immunoreactivity against anti-C52B1 antibody was observed as characteristic intranuclear spot-like positivity in the tumour cells, and some tumour cells, even in the same tissue, have immunoreactivity against this antibody[9]. In case 1, immunoreactive evaluation of re-proliferative tumour cells against anti-C52B1 antibody was difficult. However, because more than 50% of tumour cells were positive compared with Ki67 immunostaining, a diagnosis of NMC was confirmed according to criteria reported by Bishop et al.[9].

This study is the first report of NMC diagnosed using anti-C52B1 antibody in oral poorly differentiated squamous cell carcinoma. The limitation of this study was retrospective on a small number of cases at a single institute. In our department, the modality of surgery alone was basically preferred for the treatment of patients with oral cancer; however, patients who hesitated to consent to surgical intervention or for whom surgery was not available because of a busy schedule were selected for neoadjuvant chemotherapy (NAC). However, during the period of NAC, patients were encouraged to undergo surgery after completion of chemotherapy. In this study, since the NAC was

performed in some cases, the NAC might affect the treatment outcome. However, adequate surgical resection and postoperative radiation may improve prognosis, and further investigation based on a larger number of cases at multiple centres is needed.

In conclusion, the incidence of NMC in the oral cavity was 7.4% with high malignancy and poor prognosis. More cases of NMC are needed to establish a treatment strategy.

Funding

None.

Competing interests

None.

Ethical approval

The study protocol was approved by the Ethics Committee of Shinshu University School of Medicine (No. 3231).

Patient consent

Not required.

References

1. French CA. NUT midline carcinoma. *Cancer Genet Cytogene* 2010;**203**:16–20.
2. French CA, Kutok JL, Faquin WC, Toretsky JA, Antonescu CR, Griffin CA, Nose V, Vargas SO, Moschovi M, Tzortzatou-Stathopoulou F, Miyoshi I, Perez-Atayde AR, Aster JC, Fletcher JA. Midline carcinoma of children and young adults with NUT rearrangement. *J Clin Oncol* 2004;**22**(20):4135–9.
3. Bauer DE, Mitchell CM, Strait KM, Lathan CS, Stelow EB, Luer SC, Muhammed S, Evans AG, Sholl LM, Rosai J, Giraldi E, Oakley RP, Rodriguez-Galindo C, London WB, Sallan SE, Bradner JE, French CA. Clinicopathologic features and long-term outcomes of NUT midline carcinoma. *Clin Cancer Res* 2012;**18**:5773–9.
4. French CA. Pathogenesis of NUT midline carcinoma. *Annu Rev Pathol* 2012;**7**:247–65.
5. Chau NG, Hurwitz S, Mitchell CM, Aserlind A, Grunfeld N, Kaplan L, Hsi P, Bauer DE, Lathan CS, Rodriguez-Galindo C, Tishler RB, Haddad RI, Sallan SE, Bradner JE, French CA. Intensive treatment and survival outcomes in NUT midline carcinoma of the head and neck. *Cancer* 2016;**122**:3632–40.
6. Suzuki S, Kurabe N, Minato H, Ohkubo A, Ohnishi I, Tanioka F, Sugimura H. A rare Japanese case with a NUT midline carcinoma in the nasal cavity: a case report with immunohistochemical and genetic analyses. *Pathol Res Pract* 2014;**210**:383–8.
7. Matsuo M, Nishijima T, Koike K. A case of NUT (nuclear protein in testis) midline carcinoma of the ethmoid sinuses. *Nippon Jibiinkoka Gakkai Kaiho* 2017;**120**:1337–42.
8. Haack H, Johnson LA, Fry CJ, Crosby K, Polakiewicz RD, Stelow EB, Hong SM, Schwartz BE, Cameron MJ, Rubin MA, Chang MC, Aster JC, French CA. Diagnosis of NUT midline carcinoma using a NUT-specific monoclonal antibody. *Am J Surg Pathol* 2009;**33**(7):984–91.
9. Bishop JA, Westra WH. NUT Midline carcinomas of the sinonasal tract. *Am J Surg Pathol* 2012;**36**(8):1216–21.
10. Edge SB, Byrd DR, Compton CC, Fritz AG, Greene FL, Trotti A, editors. *AJCC Cancer Staging Manual*. 7th ed. New York: Springer; 2010.
11. Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, Dancey J, Arbuck S, Gwyther S, Mooney M, Rubinstein L, Shankar L, Dodd L, Kaplan R, Lacombe D, Verweij J. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). *Eur J Cancer* 2009;**45**:228–47.
12. French CA, Ramirez CL, Kolmakova J, Hickman TT, Cameron MJ, Thyne ME, Kutok JL, Toretsky JA, Tadavarthy AK, Kees UR, Fletcher JA, Aster JC. BRD-NUT oncoproteins: a family of closely related nuclear proteins that block epithelial differentiation and maintain the growth of carcinoma cells. *Oncogene* 2008;**27**:2237–42.
13. Schwartz BE, Hofer MD, Lemieux ME, Bauer DE, Cameron MJ, West NH, Agoston ES, Reynoird N, Khochbin S, Ince TA, Christie A, Janeway KA, Vargas SO, Perez-Atayde AR, Aster JC, Sallan SE, Kung AL, Bradner JE, French CA. Differentiation of NUT midline carcinoma by epigenomic reprogramming. *Cancer Res* 2011;**71**(7):2686–96.
14. Kubonishi I, Takehara N, Iwata J, Sonobe H, Ohtsuki Y, Abe T, Miyoshi I. Novel t(15;19)(q15;p13) chromosome abnormality in a thymic carcinoma. *Cancer Res* 1991;**51**:3327–8.
15. Parikh SA, French CA, Costello BA, Marks RS, Dronca RS, Nerby CL, Roden AC, Peddareddigari VG, Hilton J, Shapiro GI, Molina JR. NUT midline carcinoma an aggressive intrathoracic neoplasm. *J Thorac Oncol* 2013;**8**:1335–8.
16. French CA. Demystified molecular pathology of NUT midline carcinomas. *J Clin Pathol* 2010;**63**:492–6.
17. Mills AF, Lanfranchi M, Wein RO, Mukand-Cerro I, Pilichowska M, Cowan J, Bedi H. NUT midline carcinoma: a case report with a novel translocation and review of the literature. *Head Neck Pathol* 2014;**8**:182–6.
18. Stelow EB, Bellizzi AM, Taneja K, Mills SE, Legallo RD, Kutok JL, Aster JC, French CA. NUT rearrangement in undifferentiated carcinomas of the upper aerodigestive tract. *Am J Surg Pathol* 2008;**32**:828–34.
19. Wasserman JK, Purgina B, Sekhon H, Gomes MM, Lai C. The gross appearance of a NUT midline carcinoma. *Int J Surg Pathol* 2016;**24**(1):85–8.
20. Harada Y, Koyama T, Takeuchi K, Shoji K, Hoshi K, Oyama Y. NUT midline carcinoma mimicking a germ cell tumor: a case report. *BMC Cancer* 2016;**16**:895–900.

Address:
Shin-ichi Yamada
Shinshu University
Department of Dentistry and Oral Surgery
Asahi 3-1-1
Matsumoto
3908621
Japan
Tel.: +81 026 337 2675
E-mail: yshinshin@shinshu-u.ac.jp