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Secondary Squamous Cell Carcinoma of the Oral Cavity in Young Adults after Hematopoietic Stem Cell Transplantation for Leukemia: Report of Two Cases with Human Papillomavirus Infection

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Abstract: Two cases of secondary oral squamous cell carcinoma (SCC), which developed in recipients of hematopoietic stem cell transplantation (HSCT) for leukemia, are reported. The first patient underwent allogeneic HSCT for chronic myelogenous leukemia at 32 years of age. He suffered from chronic graft-versus-host-disease (GVHD) of the oral mucosa after HSCT, and has subsequently received immunosuppressive therapy. He experienced metachronous multiple SCCs in the maxillary gingiva and the dorsum of the tongue at 36 years and 40 years of age, respectively. The second patient received autologous HSCT for acute myelogenous leukemia at 22 years of age, and she did not experience GVHD after transplantation. SCC developed in the lateral border of the tongue at 27 years of age. PCR analysis detected both HPV16 and HPV18 in the tongue tumor of the first patient, and only HPV18 in that of the second patient, suggesting that the infection of high-risk HPVs was possibly involved in the development of post-HSCT oral cancers in these patients. Since risk factors for post-HSCT oral SCC are not yet well recognized, long-term close follow-up is necessary for the early detection of secondary oral cancers in all transplant recipients.

Key words: Oral squamous cell carcinoma, Hematopoietic stem cell transplantaion, Human papillomavirus, Secondary malignancy, Leukemia

Introduction

One of the long-term complications associated with hematopoietic stem cell transplantation (HSCT) is the development of secondary malignancies. These fall into three general categories: solid tumors, hematologic malignancies (primarily therapy-related myelodysplastic syndrome (MDS) and acute myelogenous leukemia (AML)), and post-transplant lymphoproliferative disorder. Increased risk of post-transplant solid tumors has been confirmed in the oral cavity, liver, brain and cervix[1–3]. The oral cavity is one of the most prevalent

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sites of post-HSCT solid tumors, especially in patients with chronic graft-versus-host-disease (GVHD) after transplantation[4], who have an increased risk 10.2 to 17.3 times that of the general population[1–3]. GVHD is a syndrome characterized by alloreactivity and immunodeficiency. Immunodeficiency is further aggravated by prolonged immunosuppressive therapy of chronic GVHD, which is administered for several years. Chronic GVHD can influence tissue repair and increase the chance of oncogenetic virus infection, thereby enhancing the risk of tumor evolution.

Tobacco and alcohol consumption are well-established risk factors for oral cancer. However, a small proportion of oral cancers occur in nonsmokers and nondrinkers. In a case-control study[5], the presence of human papillomavirus (HPV) in the oral cavity was associated with an increased risk of oral cavity or oropharyngeal cancer, independent of tobacco or alcohol exposure. Furthermore, a meta-analysis of 94 reports including 4,860 oral SCCs conducted by Miller *et al.*[6] has shown a significant association between infection with high-risk HPV and oral SCC.

In this report, we present two cases of SCC arising in the oral cavity of young patients, who had undergone HSCT for the treatment of leukemia. In both the tumors, the presence of high-risk HPV DNA was demonstrated by polymerase chain reaction (PCR) analysis. Risk factors for the development of secondary oral cancers in post-transplant patients are discussed.

Case Reports

Case 1: The patient was a 40-year-old male who underwent allogeneic HSCT for the treatment of chronic myelogenous leukemia at 32 years of age. He had no history of smoking, and alcohol intake was restricted to social occasions.

In June 1991, allogeneic HSCT from an unrelated donor matched for HLA-phenotype was carried out after induction by total body irradiation (TBI) and chemotherapy using cytarabine (Ara-C) and cyclophosphamide. Seven months later, he developed chronic GVHD involving the skin, oral mucosa, gastrointestinal tract and liver, and oral administration of cyclosporine and prednisolone was started.

In May 1995, the patient underwent resection of a squamous cell carcinoma of the right maxillary gingiva following radiotherapy of 30 Gy at another hospital. No detailed information was available on the treatment and histopathological features of this tumor.

In July 1999, he was referred to the Department of Dentistry and Oral Surgery, Oita Prefecture Hospital by his medical doctor because of tongue tumors. He had exfoliated dermatitis, as well as pigmentation, of the facial skin. Oral examination revealed atrophy of the lingual papillae and diffuse white patches on the dorsal surface of the tongue, where two verrucous lesions measuring 15 × 12 mm and 9 × 9 mm were observed (Fig. 1A). Erythematous change with scattered white patches was noted throughout the palatal and buccal mucosa. Incisional biopsy from the tongue tumors showed a verrucous carcinoma, and resection of the tumors was carried out under general anesthesia in September 1999. Histopathologically, the excised specimen revealed verrucous proliferation of a well-differentiated squamous epithelial tumor (Fig.1B, 1C) , of which the final diagnosis was verrucous carcinoma. Neither epithelial dysplasia nor koilocytosis was observed in the epithelium adjacent to the carcinoma.

In April 2001 and April 2003, the patient experienced recurrence of verrucous lesions of the dorsal tongue and underwent resection of the tumors under general anesthesia. The pathological diagnosis of both lesions was a verrucous carcinoma. After August 2003, he failed to continue with follow-up examinations at our hospital.

By consensus PCR and Southern blot analysis, both HPV16 and HPV18 DNA were detected in the sample from the tongue tumor resected at the first operation in September 1999 (Fig. 3).

Case 2: The patient was a 27-year-old female with acute myelogenous leukemia diagnosed at 22 years of age. She reported no history of smoking and indulged only in occasional alcohol use.

In September 1994, she received an autologous peripheral blood stem cell transplantation after induction treatment consisting of enocitabine, daunorubicin, mercaptopurine and prednisolone (the BHAC-DMP regimen). Subsequently, consolidation treatment consisted of cytarabine (Ara-C) , mitoxantrone and etoposide was done. Thereafter, she has remained stable with no evidence of a relapse of the leukemia until now.

In December 1999, the patient had an irritated feeling on the left lateral tongue when eating and visited Oita University Hospital. On examination, a white patch measuring 8 × 10 mm was noted on the left bor-

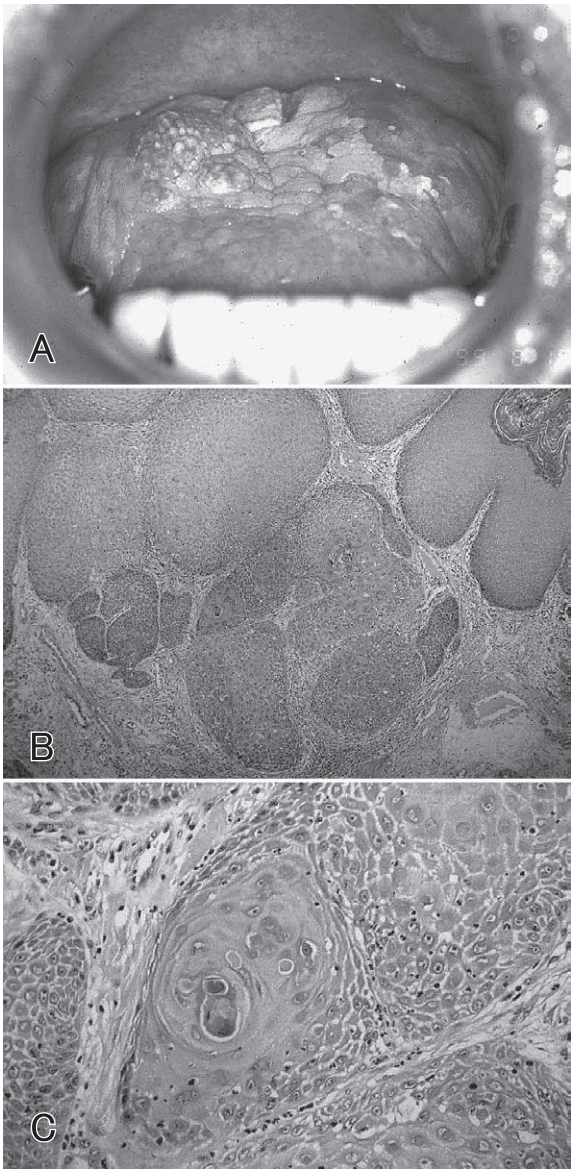

Fig. 1 Intraoral photograph of Case 1 shows verrucous tumors accompanied with white patches on the dorsum of the tongue (A). Excised material reveals a proliferation of well-differentiated squamous epithelial tumors (B, C). B: ×40, C: ×200, H-E stain

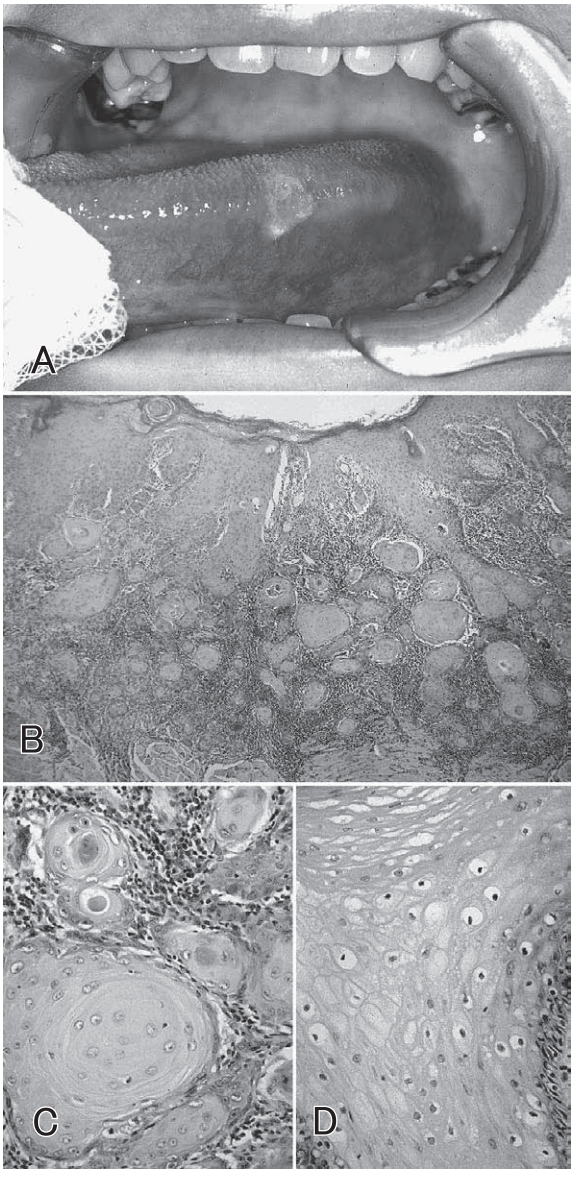

Fig. 2 Intraoral photograph of Case 2 shows a white patch on the lateral border of the tongue (A). Histologically, invasive tumor nests of well-differentiated oral SCC are observed (B, C). Koilocytosis was observed in the non-neoplastic epithelium adjacent to the carcinoma (D). B: ×40, C: ×200, D: ×200, H-E stain

der of the tongue (Fig. 2A). No submucosal induration accompanied the lesion. Since cytological diagnosis by exfoliative cytologic examination was Class II and the patient rejected excision of the lesion, a periodic follow-up was conducted once a month with the clinical diagnosis of leukoplakia of the tongue. The patient moved to another prefecture in Japan in February 2000 and we referred her to a university hospital for a follow-up of her tongue lesion.

In May 2000, the patient agreed to undergo surgical treatment of the lesion, because no spontaneous regression was observed. The lesion was excised with a safety margin of 5 mm under local anesthesia. Histopathological examination showed an invasive SCC of the well-differentiated type (Fig. 2B, 2C). Koilocytosis, but no dysplastic change, was observed in the non-neoplastic

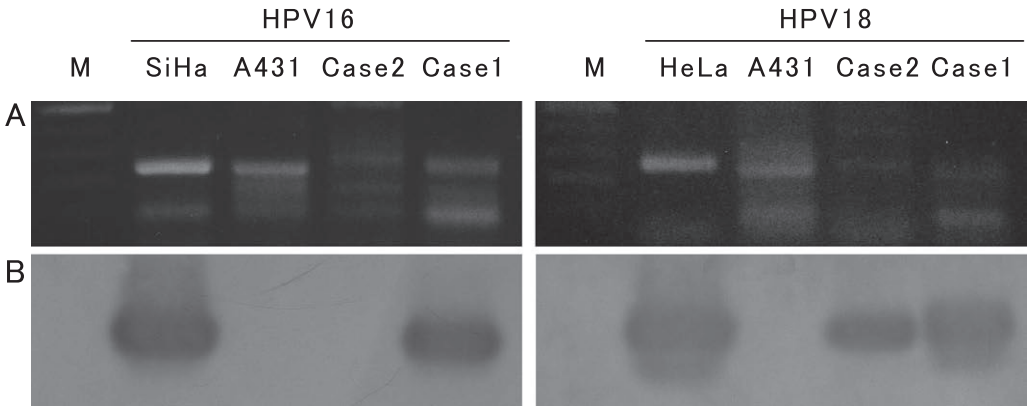

Fig. 3 Consensus PCR for the HPV16 and HPV18 E6/E8 open reading frames (A) and Southern blot analysis (B). HPV16 DNA was detected only in Case 1, and HPV18 was detected in both Case 1 and Case 2. (SiHa: a positive control for HPV16 DNA, HeLa: a positive control for HPV18 DNA, A431: a negative control, M: molecular marker)

epithelium adjacent to the carcinoma (Fig. 2D). No regional or distant metastatsis was detected by radiological examinations. The tumor was classified as stage I (T1N0M0). There has been no evidence of recurrence after the operation.

Consensus PCR and Southern blot analysis showed a positive reaction for HPV18, but not for HPV16, in the excised material (Fig. 3).

Materials and Methods

Consensus PCR and Southern blotting for HPV16 and HPV18 DNA: DNA was extracted from paraffin-embedded sections of resected tumors. In Case 1, paraffin sections of the tongue tumor resected in September 1999 were used, because sections of the maxillary tumor were not available. For each case, five 10-μm thick sections were manually microdissected under a stereomicroscope to discard the non-cancerous areas. The cancerous areas remaining on the slide glasses were processed to extract total DNA with the DNA PS Isolator Kit® (Wako Pure Chemical Industries, Ltd., Japan). Consensus primers for E6/E7 open reading frames were used to amplify the HPV DNA. The sets of primers used for the detection of HPV16 and HPV18 are as shown below.

Consensus primers for HPV DNA:

HPV16: sense primer:
5'-TGTCAAAAACCGTTGTGTCCAGAAGAAAA-3'
antisense primer:
5'-GAGCTGTCGCTTAATTGCTC-3'

HPV18: sense primer:
5'-TGTCAAAAACCGTTGTGTCCAGAAGAAAA-3'
antisense primer:
5'-TCTGAGTCGCTTAATTGCTA-3

PCR amplifications for each primer set were performed for 40 cycles consisting of denaturation at 94℃ for 1 minute, annealing at 55℃ for 2 minutes and elongation at 72℃ for 2 minutes. The PCR products were separated in 2% agarose gel by electrophoresis, and transferred to a nylon membrane. The genotype of HPV was confirmed by Southern blot hybridization analysis using the oligonucleotide probes for HPV16 and HPV18 as shown below.

Oligonucleotide probes:

HPV16: 5'-TGCAACAAGACATACATCGACCGGTCCACCGAC-3'

HPV18: 5'-TCGAGCACGAATGGCACTGGCCTCTATAGTGCCCAG-3'

Positive controls for HPV16 and HPV18 were cellular DNA from the SiHa and HeLa cell lines, respectively. A negative control for no HPV DNA integrated was cellular DNA from the A431 cell line. Signals were detected by use of the DIG Luminescent Detection Kit (Boehringer Mannheim Biochemica, Germany).

Discussion

The risk of solid tumors after HSCT has been reported to increase by 2- to 8-fold compared to that of the general population[1–3]. The oral cavity is one of the most prevalent sites for secondary solid tumors, and there is a 10.2- to 17.3-fold increased risk of oral SCC after HSCT[1–3]. The risk factors for development of secondary oral cancers after HSCT remain controversial. Al-

though a significant association between conditioning with TBI and subsequent MDS and AML has been reported[7], the impact of TBI on the incidence of subsequent solid tumors has varied among reports with significant risk[1,2] or no risk[3]. Younger age at the time of HSCT was found to be a risk factor of post-transplant solid tumors[1,3]. A recent large study reported an increased risk of SCC of the oral cavity and skin in patients who developed chronic GVHD after HSCT and received prolonged immunosuppressive therapy[4]. Patients with chronic GVHD exhibit persistent inflammation of the involved organs and prolonged immunosuppression may compromise immune surveillance, thereby enhancing the risk of tumor evolution.

Both of our patients were younger than patients with a typical oral SCC, which generally occurs in the 6th and 7th decades. Oral SCC in young adults has been recognized as a clinical entity, with potential risk factors including HPV infection, immunosuppression, and genetic susceptibility[8]. High-risk subgroups of the HPV virus, such as HPV16 and HPV18, are known to be tumorigenic for human epithelium through the actions of two viral oncogenes, E6 and E7, which interfere with cellular growth regulatory proteins[9]. The E6 protein can inactivate p53 by targeting the protein for ubiquitination and degradation[10]. E7 interacts with pRB and inactivates this protein[11]. When data from 94 reports, which analyzed 4,860 oral SCC samples, were included in a recent meta-analysis[6], HPVs were 4.7 times more likely to be detected in oral SCC than in normal mucosa. The possibility of detecting high-risk HPVs (HPV16 and 18) in oral SCC was 2.8 times greater than that of low-risk HPVs, thereby providing evidence that oral infection with HPV, particularly with high-risk genotypes, is a significant risk factor for oral SCC.

The route of HPV transmission to the oral cavity is not completely understood. Kojima *et al.*[12] reported that the rate of HPV positivity in the oral cavity was approximately 50% in 3- and 5-year-old children, probably due to vertical transmission from mother to newborn, inoculation from cutaneous warts and other person-to-person contacts. In adults, HPV positivity in the oral cavity was higher than in children and adolescents[13]. The higher rate of HPV positivity in adults is most likely caused by an accumulated lifetime exposure to the viruses, with the added risk of sexual activity. Even in transplant recipients, HPV infection was considered to be a causative agent for oral SCC[14–16]. Bradford *et al.*[14] observed prominent koilocytosis in oral SCC specimens from transplant recipients. Histopathologically, koilocytic changes in oral SCC are not predominantly found. In contrast, these changes in uterine cervix SCC are identified more frequently, suggesting viral infection. Using PCR analysis, Zhang *et al.*[16] showed increased infection with either HPV16 or HPV18 in post-transplant oral SCC patients. On the other hand, Abdelsayed *et al.*[17] and Szeto *et al.*[18] failed to detect HPV DNA in oral SCC associated with chronic GVHD after HSCT. Although HPV infection may not be sufficient to cause oral cancer, it probably plays a role as a cofactor for the development of post-transplant oral SCC.

In the present report, the first patient (Case 1) underwent TBI as the conditioning regimen and had persistent symptoms of chronic GVHD following allogeneic HSCT. He has received immunosuppressive therapy to treat chronic GVHD for over eight years. During this period, he experienced metachronous multiple oral cancers in the maxillary gingiva and the dorsum of the tongue. It has been reported that the dorsum of the tongue is a very rare site for the occurrence of SCC, accounting for 3 to 5% of lingual SCC[19]. SCCs occurring on the dorsum of the tongue are characterized by the existence of predisposing conditions as follows: longstanding lichen planus[20], median rhomboid glossitis[21], Farconi's anemia[22] and chronic GVHD of the oral mucosa[18]. Since the patient used neither tobacco nor alcohol, other multiple risk factors such as TBI, chronic GVHD, long duration of immunosuppressive therapy and high-risk HPV infection were most likely involved in the development of the post-transplant oral cancers.

The second patient (Case 2) had no potential risk factors, other than HPV infection, for the development of oral cancer. She received multiple drug chemotherapy, but no irradiation, for marrow ablation prior to autologous HSCT and has not experienced acute or chronic GVHD following transplantation. In this patient, HPV infection possibly exerted a cocarcinogenic effect on the genetic damage caused by multiple drug chemotherapy.

Although the risk of secondary leukemia and lymphoma may not extend beyond the first decade after HSCT, that of secondary solid tumors will increase with long-term follow-up[23]. The cumulative incidence of developing solid tumors did not plateau and continued to increase even 20 years after HSCT[3]. Previous reports

highlight the susceptibility of patients with chronic GVHD or irradiation to the development of secondary oral SCC[2,4], but secondary oral cancers develop even in transplant patients who do not have such risk factors, as in Case 2 of the present report. Since risk factors for secondary oral cancer are not yet well recognized, long-term close follow-up is necessary for the early detection of secondary oral SCC in all transplant recipients.

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