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Case Report

A Case of Malignant Transformation of Recurrent Respiratory Papillomatosis That Was Effectively Treated with Pembrolizumab

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Papillary squamous cell carcinoma · Human papillomavirus · Immune checkpoint inhibitor · Case report

Abstract
Introduction: Recurrent respiratory papillomatosis (RRP) is a benign tumor caused by the human papillomavirus that mainly occurs in the larynx; the tumor is known to show malignant transformation in a small percentage of cases. In this report, we describe a case of rapidly growing cervical metastasis due to malignant transformation of RRP that was successfully treated with pembrolizumab. ***Case Presentation:*** A 79-year-old man diagnosed as having laryngeal papilloma was referred to our department, and we resected the tumor by transoral laser microsurgery (TLM). Postoperative histopathology showed papillary lesions with mild cellular atypia, suggestive of papilloma. Six years later, the patient visited our hospital again with worsening hoarseness of the voice and an enlarged papillary lesion of the larynx. We performed a second TLM and postoperative histopathological examination revealed a papillary squamous cell carcinoma. In the early postoperative period, the patient developed a recurrence at the primary site and received radiotherapy (70 Gy). Three months after the completion of radiotherapy, a rapidly enlarging metastatic lymph node was observed in the right cervical region, and we performed neck dissection. Five months after the surgery, a rapidly enlarging mass appeared in the anterior cervical subcutaneous region, and the patient received palliative radiation. Since an immunohistochemical examination of a specimen obtained at the time of neck dissection showed high PD-L1 expression, with a combined positive score of 100, the patient was initiated on treatment with pembrolizumab 1 month later. Although the patient

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developed fever and a skin rash as adverse events, the tumor disappeared almost entirely within 3 weeks. Thereafter, we have observed no evidence of recurrence for over 1 year. ***Conclusion:*** The PD-1/PD-L1 pathway has been implicated in the tumorigenesis of RRP. This pathway may also play an important role in the malignant transformation of RRPs. Therefore, evaluation of tumor PD-L1 expression is recommended in these cases.

Introduction

Recurrent respiratory papillomatosis (RRP) is a disorder characterized by aerodigestive tract papillomatosis manifesting clinically as severe voice disturbance and airway obstruction [1, 2]. RRP is reported to occur at an incidence of 1.8 per 100,000 persons in adults [3], and malignant transformation reportedly occurs in 1.6–4.0% of cases [4, 5]. Although surgical resection of the tumor is the standard of care, recurrence and the morbidity associated with both the disease itself and the repeated surgical interventions remain problematic [6]. In most cases, RRP is caused by low-risk human papillomavirus (HPV) types 6 and 11 [7]. Current literature supports polarization of the adaptive immune response to a T-helper type 2 (Th2)-like or T-regulatory phenotype, driven by a complex interplay between innate immunity, adaptive immunity and the HPV 6/11 proteins [8]. Some reports suggest that the majority of RRP specimens show PD-1-positive infiltrating immune cells and PD-L1 expression on both the squamous papilloma cells and infiltrating immune cells [9]. There is a need to find effective therapies to reduce the burden of repeated recurrences and surgeries in patients with RRP, as also for patients with malignant transformation of RRP. Herein, we describe a case of rapidly progressive malignantly transformed RRP, which was successfully treated with the anti-PD-1 agent, pembrolizumab. The CARE Checklist has been completed by the authors for this case report, attached as online supplementary material (for all online suppl. material, see https://doi.org/10.1159/000540009).

Case Presentation

The endoscopic and histopathological findings of our case are summarized in Figures 1 and 2, respectively. A 79-year-old man without any significant past medical history was referred to our hospital with a lesion in his right vocal fold detected by esophagogastroduodenoscopy during a medical checkup. Laryngoscopy performed at our hospital revealed protruding papillary lesions on the larynx bilaterally, predominantly on the right side (Fig. 1a). As biopsy revealed a papilloma, we resected the right-sided tumor by transoral laser microsurgery (TLM). Postoperative histopathology showed papillary lesions with mild cellular atypia, suggestive of papilloma (Fig. 2a). Four years after the initial surgery, the patient presented with a recurrent tumor in the same position on the larynx (Fig. 1b). We performed a repeat biopsy, which showed no evidence of malignancy (Fig. 2b). Therefore, we transferred the patient to the local clinic for follow-up. However, 6 years after his first visit (or 2 years after the last biopsy), he visited our hospital again with worsening hoarseness of the voice and an enlarged papillary lesion spreading over the larynx, mainly over the right vocal cord (Fig. 1c). A biopsy at this time revealed a papillary tumor showing severe atypia (Fig. 2c). Because of the risk of development of upper airway stenosis, we performed a TLM again (Fig. 1d). Postoperative histopathology revealed a papillary squamous cell carcinoma (PSCC).

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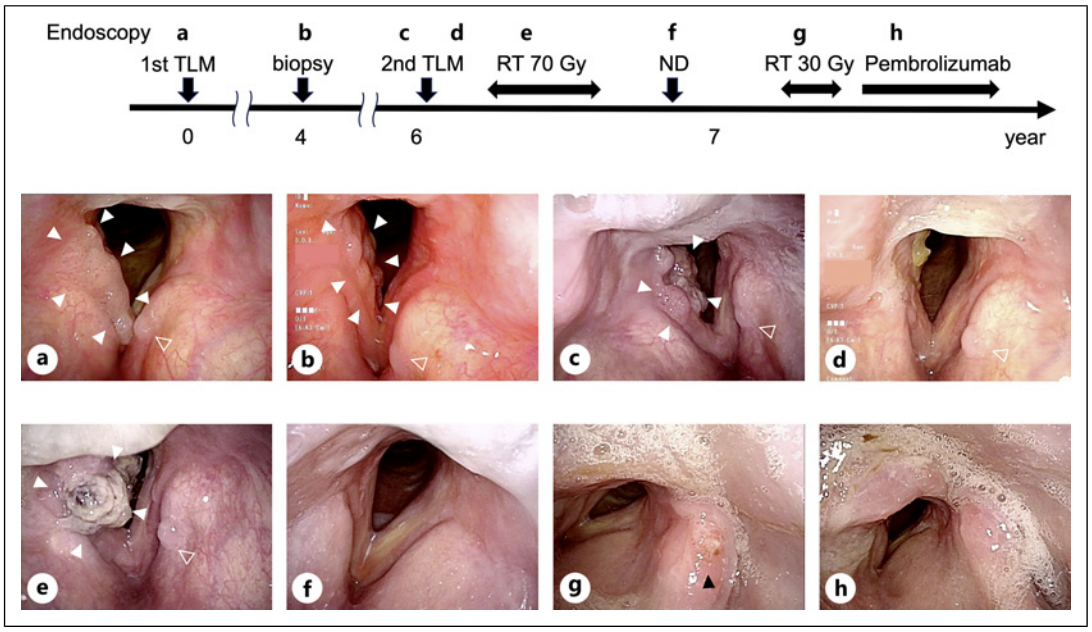

Fig. 1. Transition of endoscopic findings of larynx. **a** Just before the first TLM. A laryngeal papillary tumor is seen on the right side (white filled arrowhead). A small lesion is also found on the left false vocal cord (white clear arrowhead). **b** Four years after the initial TLM. Recurrence of the right-sided laryngeal tumor is seen (white filled arrowhead), while the lesion in the left false vocal cord shows minimal change (white clear arrowhead). **c** Just before the second TLM. The right laryngeal tumor has grown large enough to cause narrowing of the airway (white filled arrowhead), while the left laryngeal tumor shows minimal change (white clear arrowhead). **d** Just after the second TLM. The right-sided tumor has been almost entirely resected, while the left tumor has not been resected. **e** One month after the second TLM. The right-sided tumor has rapidly regrown (white filled arrowhead), while the left-sided tumor shows minimal change (white clear arrowhead). **f** Three months after the RT. The tumors on both sides have disappeared. **g** Ten months after completion of the RT. A new small papillary lesion has appeared on the left arytenoid (black filled arrowhead). **h** One month after the start pembrolizumab. The small tumor on the left side is no longer seen. ND, neck dissection; RT, radiation therapy; TLM, transoral laser microsurgery.

He was diagnosed with T2N0M0 glottic laryngeal cancer. Since the patient presented with a rapid recurrence at the primary site at only 1 month after the surgery (Fig. 1e), we administered radiation therapy (70 Gy), which eradicated the tumors in the right vocal cord as well as the left false vocal cord (Fig. 1f). Three months after the completion of radiotherapy, the patient presented again with a rapidly enlarging metastatic lymph node in the right cervical region within the irradiated area (Fig. 3a), and we performed right neck dissection (Fig. 3b). Postoperative histopathology revealed metastasis from the PSCC (Fig. 3c). The tumor was later found to show strong PD-L1 expression, with a combined positive score (CPS) of 100 (Fig. 3d). Five months after the neck dissection, the patient presented with a rapidly enlarging mass again in the anterior cervical subcutaneous region (Fig. 3e, f), with a new papillary lesion also found in the left arytenoid (Fig. 1g); biopsy histopathology revealed a papilloma with cellular atypia and a few koilocytotic changes (online suppl. Fig. 1). The patient then received palliative electron beam radiation (30 Gy) for the neck metastasis. Although the tumor size decreased after the irradiation, a residual tumor persisted. As described above, as the tumor PD-L1 CPS was high (100), we started the patient on the anti-PD-1 antibody, pembrolizumab, 1 month later. Fever of approximately 40°C was observed on day 1 of the drug administration, which gradually disappeared by the end of 1 week. Also, the

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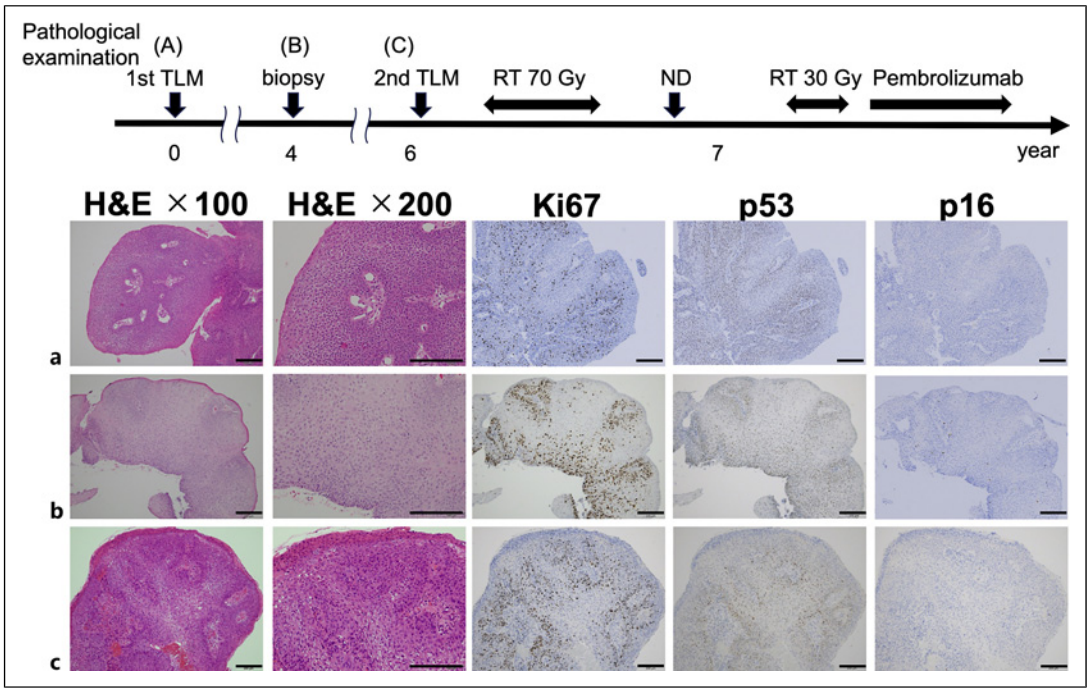

Fig. 2. Pathological findings. **a** Just before the first TLM. Papillary proliferation of highly differentiated atypical squamous epithelial cells with mildly enlarged nuclei is seen. The intracellular space is maintained. Ki67 immunohistochemical staining (IHC) shows positive cells in all areas of the tumor other than the parabasal layer. p53 IHC suggests wild-type p53 expression. p16 IHC showing negative staining. **b** Four years after the first TLM. Although the biopsy specimen showed a similar pattern of papillary proliferation, increased cellular atypia and shortening of the intracellular distance were observed. IHC for Ki67, p53, and p16 showed similar trends to those observed in the previous specimens. **c** Just before the second TLM. Severely atypical cells with enlarged and inconsistently sized nuclei are observed in all the cell layers in the stratified squamous epithelium showing papillary proliferation. IHC for Ki67, p53, and p16 showed similar trends to those in the previous specimens. All scale bars represent 200 μm. ND, neck dissection; RT, radiation therapy; TLM, transoral laser microsurgery.

patient developed a skin rash about 10 days after the start of pembrolizumab treatment, which also gradually disappeared after a few weeks. On his first visit to us after 3 weeks of treatment with pembrolizumab, the tumor had disappeared nearly entirely. The new papilloma on the left arytenoid also disappeared in the following month. Although the patient experienced some adverse events as described above, pembrolizumab seemed to be very effective against the tumor so we continued the patient on the same drug. After 3 months, a physical examination and CT revealed a complete response (Fig. 3g, h), and the patient showed no evidence of tumor recurrence for over 1 year.

Discussion

We have reported a case of PSCC resulting from malignant transformation of RPP, that was successfully treated with the anti-PD-1 antibody, pembrolizumab. RRP is strongly associated with HPV 6/11 infection. While approximately 1% of the population is known to harbor HPV 6/11 DNA in the larynx [10], a very small percentage of HPV-exposed individuals develop RRP [11]. This suggests that immune dysfunction may contribute to the development

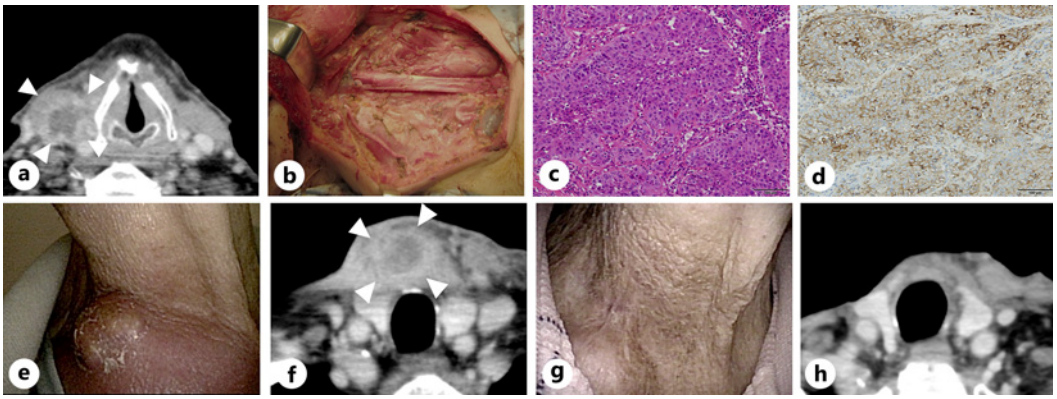

Fig. 3. Cervical metastasis. **a** Three months after the radiation therapy. An enlarged cervical lymph node with central necrosis (white arrowhead) was detected by CT. **b** Cervical lymph nodes were removed by neck dissection. **c** Specimen of an excised lymph node. Proliferating stratified squamous epithelial cells are seen with enlarged nuclei. **d** IHC for PD-L1. The PD-L1 combined positive score (CPS) was 100. **e** Five months after the neck dissection. An anterior cervical subcutaneous metastasis is observed. **f** CT image. A metastatic node (white arrowhead) is seen on the trachea. **g** Three months after the initiation of pembrolizumab. The cervical metastasis appears to have completely disappeared. **h** CT obtained 3 months after the initiation of pembrolizumab. The image shows complete tumor response.

of RRP. One study showed that RRP patients have an increased count of a specific subset of circulating $CD4^+$ T cells that express Th2-like cytokines; therefore, the adaptive immune response in patients with RRP becomes polarized toward a Th2-like or T-regulatory phenotype [12]. There is evidence that HPV E6 protein can directly inhibit IL-2 and IL-18 expressions, which normally contribute to maintaining a balanced adaptive immune response and an HPV-specific cytotoxic T-cell function [13]. Thus, HPV-infected cells alter as well as escape from cellular immunity, resulting in the development of RRP.

In the RRP micromilieu, adaptive immunity is strongly suppressed. According to one report, the regulatory T-cell (Treg) population is increased among $CD4^+$ T cells in papilloma tumors as compared with that in the blood of patients with RRP, which could be caused by the Treg-attracting chemokine CCL17 which is differentially expressed in the papillomas [14]. It has also been reported that as compared with the finding in laryngeal tissues from individuals without RRP, laryngeal papillomas show a 145-fold greater expression of PD-L1 mRNA, and clinically normal laryngeal tissues from patients with RRP show a 93-fold greater expression of PD-L1 mRNA [14]. A recent case report suggests that anti-PD-L1 antibody may induce durable and viral-antigen-specific immunity of sufficient magnitude to control the disease in patients with RRP patients [15]. A phase II study of pembrolizumab for HPV-associated papilloma patients with laryngeal, tracheal, and/or pulmonary involvement in the USA also lends support to the effectiveness of anti-PD-1/PD-L1 antibody therapy against RRP [16]. We believe that anti-PD-1 antibody is also effective against RRPs showing malignant transformation. In our case, the patient developed high fever and skin rash only after the first anti-PD-1 treatment and the tumor rapidly disappeared. The adverse events were probably induced by the strong immune reaction.

p16 protein is an inhibitor of cyclin-dependent kinases and an important cell cycle regulator. Clinical studies in patients with head and neck cancer have revealed a strong correlation between overexpression of p16 and expression of the HPV E6/E7 protein, a marker of active HPV infection [17]. As mentioned above, RRP is known to be strongly associated with HPV 6/11 infection, but the p16 expression is not always high. According to some reports, tumor p16 marker expression was negative in 24.2% of cases of RRP [18] and

86.4% of cases of laryngeal PSCC [19], although PSCC is thought to occur not only from HPV but also from tobacco smoking and alcohol consumption [20, 21]. Tumor p16 expression was also negative in our case reported herein. Although we did not confirm infection with HPV in our patient with RRP because the cost of the testing is not covered by Japanese National Health Insurance, we suspect that the RRP and its malignant transformation in our patient was associated with HPV infection because the lesion was recurrent and multiple, and also, a few koilocytotic changes were observed in the new lesion that appeared after the radiation therapy (online suppl. Fig. 1).

In conclusion, we have reported a case of RRP with malignant transformation to PSCC, which was successfully treated with an anti-PD-1 antibody drug. PD-L1 expression should be examined in RRP patients presenting with malignant transformation of the tumor.

Statement of Ethics

This study protocol was reviewed and approved by the Research Ethics Committee of Institute of Education, Tokyo Medical, and Dental University, Approval No. C2024-007. A written informed consent was obtained from patient for publication of the details of his medical case and any accompanying images.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

Yusuke Mizuno collected the clinical data and wrote the manuscript. Ryuhei Okada collected the clinical data, wrote the manuscript, and supervised the project. Susumu Kirimura evaluated pathological findings and wrote the manuscript. Ryoichi Yoshimura and Takahiro Asakage wrote the manuscript.

Data Availability Statement

All data generated or analyzed during this study are included in this article and its online supplementary material files. Further inquiries can be directed to the corresponding author.

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