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Research Paper

Pathological features of residual head and neck cancer after near-infrared photoimmunotherapy: implications of an unfavorable tumor immune microenvironment

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ABSTRACT

Purpose: Near-infrared photoimmunotherapy has been approved in Japan for unresectable locally advanced or recurrent head and neck cancer. While this therapy theoretically induces selective necrosis of epidermal growth factor receptor-expressing tumor cells following administration of cetuximab sarotalocan sodium and irradiation with 690-nm light, residual disease is not uncommon in clinical practice. The mechanisms underlying such persistence remain poorly understood.

Methods: We examined two patients with head and neck squamous cell carcinoma who underwent salvage resection due to residual disease after four cycles of near-infrared photoimmunotherapy. Resected specimens were subjected to histopathological evaluation, including immunohistochemistry for epidermal growth factor receptor, cluster of differentiation 8, programmed cell death protein 1, and forkhead box P3 proteins.

Results: In both cases, residual tumor cells were predominantly localized in the superficial mucosal layers, while deeper layers were replaced by fibrosis. All residual tumors retained expression of epidermal growth factor receptor. Lymphocytic infiltration was sparse, with scattered cluster of differentiation 8-positive cells but few programmed cell death protein 1-positive or forkhead box P3-positive lymphocytes. These findings suggest that the tumor microenvironment surrounding residual tumors may have been unfavorable for the induction of robust antitumor immune responses.

Conclusion: Residual tumors after near-infrared photoimmunotherapy can occur despite adequate laser irradiation and epidermal growth factor receptor expression. Our observations imply that insufficient immune activation within the tumor microenvironment may contribute to treatment resistance. Further characterization of the post-photoimmunotherapy tumor microenvironment will be essential to better understand treatment mechanisms and to optimize therapeutic efficacy.

1. Introduction

Near-infrared photoimmunotherapy (NIR-PIT) has been covered by the Japanese national health insurance system since 2021 [1]. Although clinical cases have gradually accumulated and various insights have been obtained, many aspects of the treatment remain unclear. NIR-PIT involves intravenous administration of cetuximab sarotalocan sodium followed by irradiation with 690-nm laser light, which is designed to induce rapid and selective tumor cell death [2–4]. However, tumor persistence is sometimes encountered in actual clinical practice, suggesting the existence of factors allowing resistance to treatment.

Possible reasons for tumor persistence include insufficient laser irradiation or lack of epidermal growth factor receptor (EGFR) expression in tumor cells. Nevertheless, persistence has also been observed in tumors showing confirmed EGFR expression and for which irradiation was considered sufficient [5]. Such reports indicate that, in addition to laser irradiation and EGFR expression, other factors may influence therapeutic outcomes.

We encountered two cases in which tumors persisting after NIR-PIT were surgically resected. By histopathologically examining these specimens, we sought to identify factors influencing the therapeutic effect of PIT.

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2. MATERIALS AND methods

2.1. Patients

Two cases treated at Okayama University Hospital with NIR-PIT followed by surgical resection of the treated sites due to residual disease were analyzed.

Case 1 involved a 65-year-old man with recurrent hypopharyngeal carcinoma following radiotherapy, which had been administered seven years earlier. His treatment history was notable for two prior endoscopic submucosal dissections (ESD) for hypopharyngeal carcinoma performed eight and five years before the current presentation, as well as two ESD procedures for esophageal carcinoma conducted six and five years earlier. In addition, he had undergone surgical resection for oropharyngeal carcinoma four years previously. Representative images of the lesion obtained prior to the initiation of NIR-PIT are shown (Fig. 1). He received four cycles of NIR-PIT, all delivered using a frontal diffuser. Because the tumor persisted after four cycles, surgical resection of the lesion was subsequently performed.

Case 2 involved a 70-year-old man with recurrent carcinoma of the lateral oropharyngeal wall. His medical history was notable for chemoradiotherapy for hypopharyngeal carcinoma performed 11 years earlier, surgical treatment for esophageal carcinoma over the subsequent 11-year period, and three prior ESD for hypopharyngeal carcinoma conducted 10, 8, and 5 years before the current presentation. In addition, surgical resection for recurrent hypopharyngeal carcinoma had been performed 3 years earlier. Representative images of the lesion obtained prior to the initiation of NIR-PIT are shown (Fig. 2). He underwent four cycles of NIR-PIT. In the first cycle, irradiation was performed using a frontal diffuser alone, whereas both frontal and cylindrical diffusers were employed in the second, third, and fourth cycles. Because the tumor persisted after four cycles, surgical resection of the lesion was subsequently performed.

All study protocols were approved by the institutional review board of our department (approval No 2309–040). Informed consent was obtained from all patients.

2.2. Immunohistochemistry

All specimens were fixed in 10 % formalin and embedded in paraffin. Paraffin-embedded tissue blocks were sliced into 3 μm thin sections and either stained with hematoxylin and eosin or processed for immunohistochemical staining.

Immunohistochemical staining was performed using an automated BOND-III instrument (Leica Biosystems, Wetzlar, Germany) with the primary antibody of EGFR antibody (1:200, ab52894; Abcam), cluster of differentiation 8 (CD8) (1:200, 413,211; Nichirei Biosciences), programmed cell death protein 1 (PD1) (1:200, ab52587; Abcam), and forkhead box P3 (FOXP3) (1:100, ab20034; Abcam).

3. Results

3.1. Case 1

Pathological images demonstrated the residual lesion localized the intramucosal epithelium, submucosal tissue was replaced by densified fibrous tissue accompanied by a small number of lymphocytes (Fig. 3). Residual tumor cells exhibited EGFR positivity. Scattered CD8-positive lymphocytes were present in the peritumoral infiltrate, whereas PD-1-positive and FOXP3-positive lymphocytes were infrequently identified (Fig. 4).

3.2. Case 2

Residual lesion with necrosis was observed within a densified fibrous stroma accompanied by a small number of lymphocytes (Fig. 5). The residual tumor cells were EGFR-positive, and scattered CD8-positive lymphocytes were observed in the peritumoral infiltrate, whereas PD-1-positive and FOXP3-positive lymphocytes were scarce. (Fig. 6).

4. Discussion

Findings from our two squamous cell carcinoma cases suggest the presence of factors conferring resistance beyond insufficient laser irradiation and/or EGFR expression. With respect to irradiation, previous studies have reported that conventional methods may deliver inadequate light, and that increasing the laser dose may improve therapeutic efficacy without compromising safety [6]. In the present study, irradiation was performed according to such reports. In both specimens, residual tumor was observed in the superficial layers, while the deeper and adjacent tissues exhibited histological evidence of therapeutic response, suggesting that even areas of residual tumor had been sufficiently irradiated. Regarding EGFR, although preclinical studies using cell line and animal models have reported a correlation between EGFR expression levels and the therapeutic efficacy of NIR-PIT [2,3], no such relationship has been demonstrated in clinical specimens to date, whereas other studies, including the present, have reported no such correlation [6]. Further, we previously reported a case of salivary duct carcinoma treated with NIR-PIT in which, despite sufficient irradiation and EGFR expression, tumor persistence was observed [5].

In both specimens, pathological examination revealed residual tumor in the superficial layers, while deeper tumor areas disappeared and were replaced by fibrous tissue. This spatial pattern of residual disease suggests the presence of region-specific resistance, potentially related to differences in antibody distribution, tissue architecture, vascularity, or immune cell accessibility between superficial and deeper tumor compartments. Lymphocytic infiltration around the residual tumor appeared limited. We therefore performed immunohistochemistry for CD8, PD-1, and FOXP3. CD8 was selected as a marker of

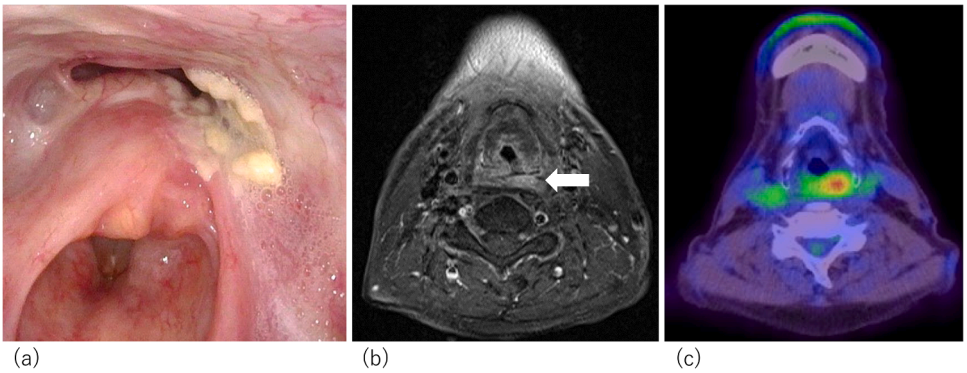

Fig. 1. Flexible laryngoscopy demonstrated a tumor in the left piriform sinus of the hypopharynx (a). Contrast-enhanced cervical magnetic resonance imaging (MRI) revealed a lesion at the site indicated by the arrow (b), with corresponding fluorodeoxyglucose uptake on FDG positron emission tomography (PET) (c).

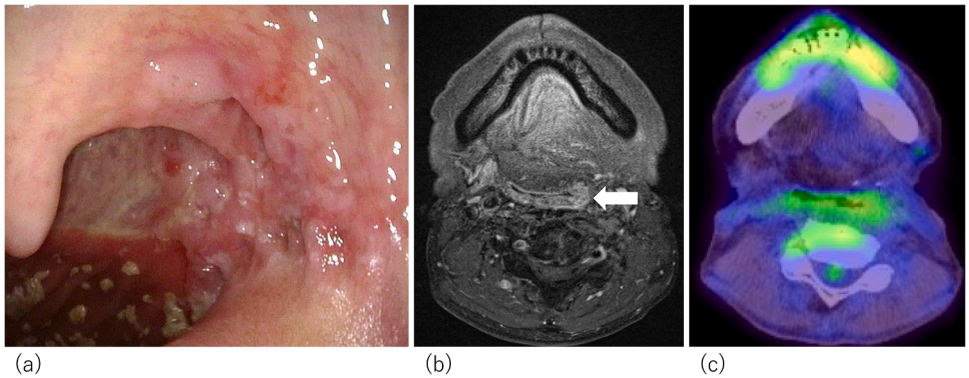

Fig. 2. Endoscopic examination revealed a mass on the left lateral wall of the oropharynx (a). Contrast-enhanced cervical MRI demonstrated a lesion at the site indicated by the arrow (b), whereas FDG-PET showed only mild uptake at the corresponding site (c).

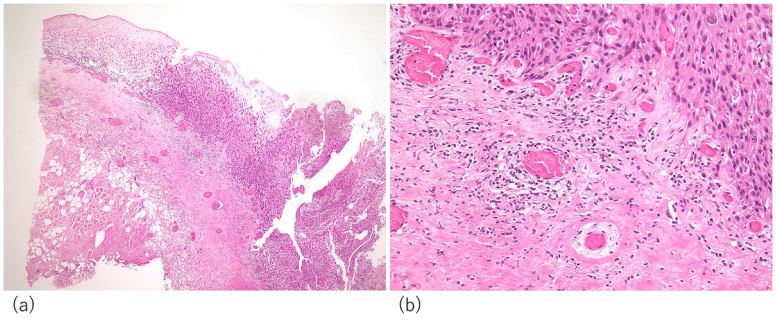

Fig. 3. Hematoxylin and eosin–stained sections of the resected specimen from Case 1. Panels (a) and (b) show low- and high-power views (× 4 and × 20 magnification, respectively). The residual lesion localized the intramucosal epithelium, submucosal tissue was replaced by densified fibrous tissue, consistent with treatment-related effect.

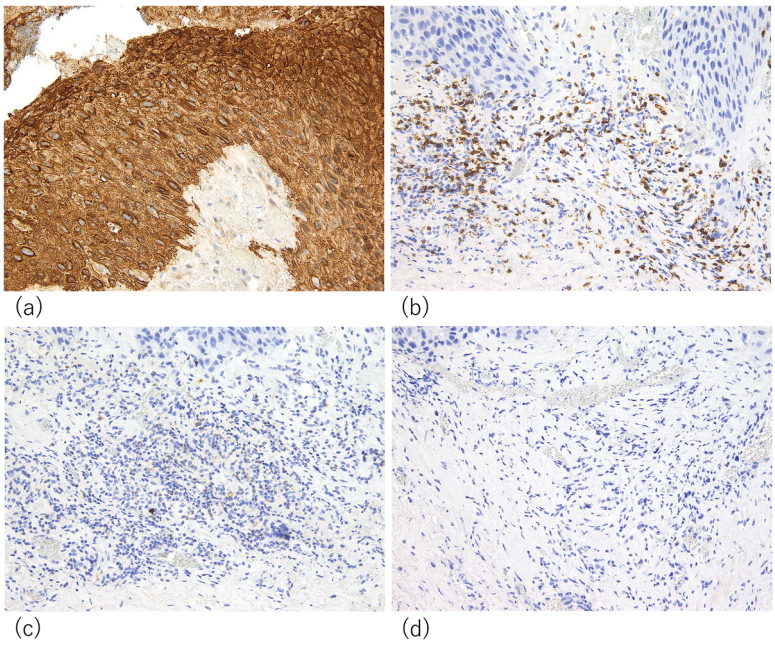

Fig. 4. Immunohistochemical findings of the resected specimen from Case 1. Panels show staining for EGFR (a), CD8 (b), PD-1 (c), and FOXP3 (d), all at × 20 magnification. Residual tumor cells exhibited EGFR positivity. Scattered CD8-positive lymphocytes were present in the peritumoral infiltrate, whereas PD-1-positive and FOXP3-positive lymphocytes were infrequently identified.

cytotoxic T lymphocytes, which are key effectors of antitumor immunity and play a central role in the cancer–immunity cycle. Given that NIR-PIT has been reported to induce immunogenic cell death and potentially enhance adaptive immune responses, evaluation of T-cell–mediated immunity was considered essential. PD-1 was included to assess the activation and exhaustion status of tumor-infiltrating T cells, while FOXP3 was examined as a marker of regulatory T cells that may modulate immune responses within the tumor microenvironment.

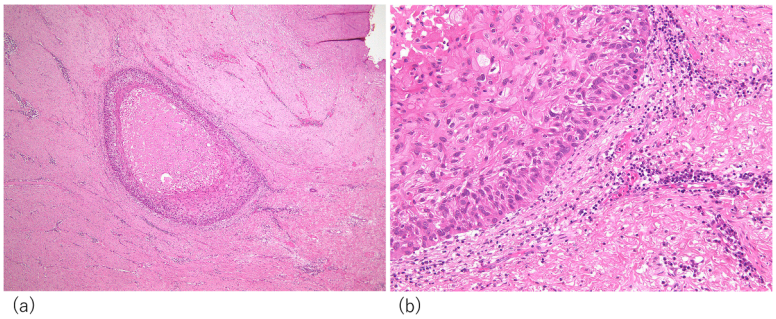

Fig. 5. Hematoxylin and eosin–stained sections of the resected specimen from Case 2. Panels (a) and (b) show low- and high-power views (× 4 and × 20 magnification, respectively). Residual lesion with necrosis was observed within a densified fibrous stroma, consistent with treatment effect.

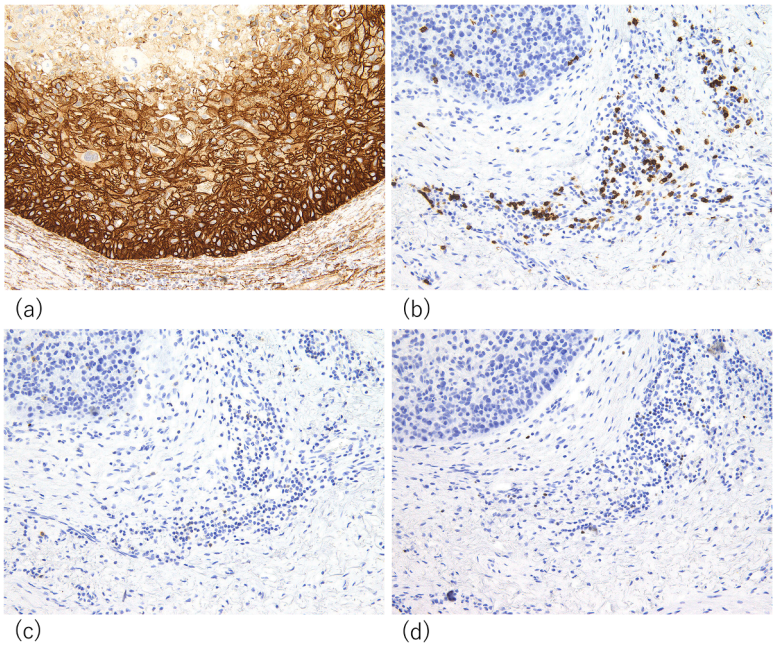

Fig. 6. Immunohistochemical findings of the resected specimen from Case 2. Panels show staining for EGFR (a), CD8 (b), PD-1 (c), and FOXP3 (d), all at × 20 magnification. Residual tumor cells were EGFR-positive, and scattered CD8-positive lymphocytes were observed in the peritumoral infiltrate, whereas PD-1-positive and FOXP3-positive lymphocytes were scarce.

Collectively, these markers allowed us to characterize not only the presence but also the functional state of T-cell–mediated immune responses surrounding the residual tumor. In both cases, lymphocytic infiltration around the residual tumor was sparse. Although CD8-positive T cells were sparsely observed around the residual tumor, PD-1–positive and FOXP3-positive lymphocytes were also infrequently identified. The low frequency of PD-1–positive cells is notable, as PD-1 expression is typically upregulated following antigen recognition and T-cell activation. Therefore, their limited presence suggests that substantial T-cell activation may not have occurred within the tumor microenvironment. Likewise, the scarcity of FOXP3-positive regulatory T cells indicates that a prominent regulatory immune response was not established. Taken together, these findings indicate that adaptive immune activation was limited rather than strongly suppressed. In other words, although T cells were physically present in the peritumoral region, the cancer–immunity cycle required for effective antitumor immune responses was likely insufficiently activated. This immune context is consistent with a “cold tumor” phenotype and may have contributed, at least in part, to resistance to NIR-PIT in these cases [7–9]. This observation further suggests that resistance to NIR-PIT may arise not only from insufficient direct tumor cell killing, but also from a failure to translate local tumor destruction into a sustained antitumor immune response.

NIR-PIT is theoretically capable of inducing immunogenic cell death via tumor cell killing [2,3,10], a process considered to promote the release of tumor antigens and facilitate subsequent adaptive immune responses. However, the establishment of sustained antitumor immunity requires coordinated progression through multiple sequential steps of the cancer–immunity cycle, including effective antigen presentation, T-cell priming, intratumoral trafficking, and local expansion of effector T cells. In the present cases, such sustained immune activation was not evident in the residual tumor areas. Importantly, dendritic cell density or activation status was not directly assessed in this study, and surgical specimens were obtained after a certain interval following NIR-PIT rather than immediately after treatment. Because immune activation and dendritic cell dynamics are inherently time-dependent processes, evaluation at this later time point may not fully reflect early immunological events triggered by NIR-PIT. Therefore, we cannot determine whether insufficient dendritic cell recruitment, impaired activation, or dysfunction at another step of the cancer–immunity cycle contributed to the limited adaptive immune activation observed. Notably, pathological examination demonstrated marked fibrosis in the treated areas. Such structural alterations of the tumor microenvironment—whether related to prior irradiation or to tissue remodeling following NIR-PIT—may

have influenced vascular integrity, immune cell trafficking, and the spatial distribution of T cells within the tumor microenvironment [11], thereby potentially limiting the translation of tumor cell destruction into a sustained adaptive immune response and contributing to residual tumor persistence.

In theory, NIR-PIT should induce cell damage and complete tumor necrosis when EGFR is expressed and irradiation is applied [2,3]. However, residual disease was observed in both cases presented here, and pathological examination indicated that the peritumoral environment may have been less conducive to the induction of immune responses. We hypothesize that while most tumor cells are destroyed by NIR-PIT, persistence occurs when effective immune responses fail. This hypothesis may also explain why smaller tumors are more likely to undergo complete necrosis and achieve cure with NIR-PIT alone, as previously reported [12]. We hypothesize that in smaller tumors, the direct cytotoxic effect of NIR-PIT is more likely to encompass the entire tumor volume, allowing tumor eradication by PIT alone. In contrast, in larger tumors, residual disease may remain after PIT, and tumor control may therefore rely on the successful induction of antitumor immune responses. Although further investigation was not feasible in the present cases, clarifying changes in the tumor microenvironment after PIT is important for elucidating the mechanisms involved and enhancing the therapeutic efficacy.

This study has several limitations. Surgical resection was performed after a certain interval following NIR-PIT, rather than immediately after treatment. Therefore, the present findings may represent residual tumor persisting after NIR-PIT; however, the possibility that the tumor initially regressed and subsequently recurred cannot be completely excluded. Clarification of this distinction would require earlier biopsy after treatment. Nevertheless, because treatment-related necrosis occurs immediately after NIR-PIT, pathological evaluation at an early time point is technically challenging, and if the tumor has completely disappeared, such biopsy would result in unnecessary invasiveness for the patient. Importantly, even if the lesions examined in the present cases represent recurrent rather than persistent disease, effective antitumor immune responses would be expected to prevent regrowth following initial tumor eradication. In this context, the immunohistochemical findings observed in our cases—namely, limited lymphocytic infiltration and a “cold tumor”–like immune microenvironment—suggest that insufficient immune activation after NIR-PIT may have contributed to tumor regrowth. Thus, confirmation that the tumors were sufficiently irradiated, expressed EGFR, and exhibited poor post-treatment immune activity represents an important insight for understanding the mechanisms underlying resistance to NIR-PIT.

5. Conclusion

We histopathologically examined resected specimens from cases with residual disease after NIR-PIT. In both cases, laser irradiation was sufficient and tumors were EGFR-positive. Pathological findings suggested that the microenvironment around residual tumors was unfavorable for immune responses. These results suggest that, in addition to the direct cytotoxic effects of NIR-PIT, post-treatment immune responses may also play an important role in determining therapeutic efficacy. To better understand the mechanisms involved and optimize the efficacy of this therapy, further investigation of the effects of NIR-PIT on the tumor microenvironment is warranted.

CRediT authorship contribution statement

Takuma Makino: Writing – review & editing, Writing – original draft, Visualization, Validation, Project administration, Methodology, Investigation, Data curation, Conceptualization. **Yasuharu Sato:** Writing – review & editing, Validation, Resources, Investigation. **Asami Nishikori:** Writing – review & editing, Validation, Resources, Investigation, Data curation. **Yuto Naoi:** Writing – review & editing, Visualization, Investigation, Data curation. **Mizuo Ando:** Writing – review & editing, Supervision, Methodology.

Declaration of competing interest

All authors declare that they have no conflict of interest.

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