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CASE REPORT

Possible involvement of stem cell factor and endothelin-1 in the emergence of pigmented squamous cell carcinoma in oral mucosa

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We present here the clinical, morphological and immunohistochemical features of a pigmented squamous cell carcinoma (SCC) in the oral mucosa of the hard palate of a 76-year-old Japanese man. He underwent a partial resection of the maxilla subsequent to radiotherapy. The tumor was typical, moderately well-differentiated SCC but had many melanocytes (melanocytosis) within it. Immunohistochemical analysis for stem cell factor (SCF) and endothelin-1, both of which are known to stimulate proliferation and differentiation of melanocytes, revealed prominent expression of both factors in the neoplastic squamous cells of the pigmented SCC, while the non-pigmented oral SCC showed little sign of either factor. These findings strongly suggest that SCF and endothelin-1 secreted by neoplasmic squamous cells are involved in the emergence of a rare variant of oral SCC.
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Case report

A 76-year-old Japanese man presented at the Tokushima University Hospital in 2005 with a slowly enlarging, painful, pigmented ulcerative tumor of the oral mucosa of the right hard palate. The patient had experienced swelling and pain for 4 months. The patient also had a past history of essential hypertension and hepatitis C. Intraoral examination revealed a 20 × 17 mm irregularly shaped, painful, partially pigmented ulcerative mass on the right hard palate (Fig. 1A). Neck lymph nodes were not palpable. Although a thoracoabdominal CT scan showed no abnormal findings, endoscopic examination of the upper gastrointestinal tract showed the early gastric adenocarcinoma (T1N0 M0). All the test values of a general examination were within normal limits.

Under the clinical diagnosis of a suspected malignant melanoma of the right hard palate, a partial resection of the maxilla was scheduled on the day following a biopsy. Under local anesthesia, an incisional biopsy of the lesion was performed, and the histopathological diagnosis was well-differentiated squamous cell carcinoma (SCC) with melanosis. The resection was postponed and pre-operative radiotherapy at 2 Gy/day to a total dose of 40 Gy was performed over a period of 28 days. A partial resection of the maxilla was then performed with a 15 mm peripheral margin, and the tumor was completely excised. Twenty-nine days later, a laparoscopy-assisted distal gastrectomy and a reconstruction by the Billroth I method were performed. Thereafter, the patient underwent an adjuvant chemotherapy with TS-1 at a dose of 80 mg/day. When last examined, the patient was free of any local recurrence or metastasis 16 months after resection.

Microscopic evaluation revealed a well-differentiated SCC. Many melanocytes were scattered in the stroma, and a considerable number of melanocytes were intermixed with the carcinoma cells (Fig. 1B,C). Immunohistochemical examination for stem cell factor (SCF) revealed a prominent and homogeneous expression of SCF in the neoplastic keratinocytes of SCC of the present case (Fig. 2A,B). In contrast to the pigmented variant of this case, the non-pigmented oral surgical SCC specimens showed little reactivity with anti-SCF antibody (Fig. 2C,D). Immunohistochemical analysis for endothelin-1 showed a high expression of endothelin-1 in the neoplastic keratinocytes of the cancer cell nests of the present case (Fig. 3A,B). In contrast, a weaker immunoreaction for endothelin-1 was noted in the neoplastic keratinocytes of the any other control cases (Fig. 3C,D).

Next, to examine whether the melanocytes scattered in the pigmented SCC of the present case are in the

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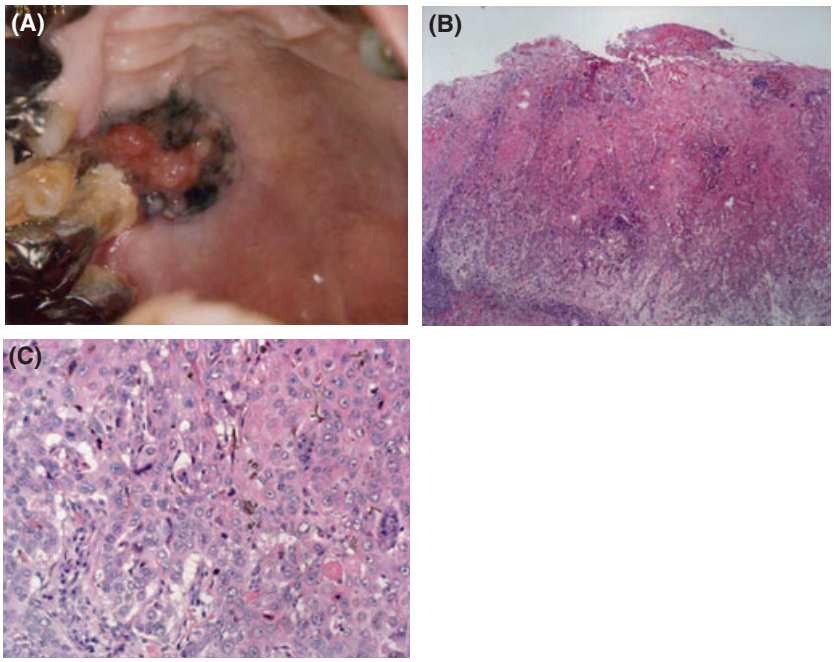

Figure 1 (A) Gross appearance of the present case of pigmented squamous cell carcinoma (SCC) in the oral mucosa of the right hard palate, revealing a 20 × 17 mm irregularly shaped, partially pigmented uncreative lesion. (B) Light microscopic appearance of the pigmented SCC of the hard palate. The lesion was composed of moderately well-differentiated SCC (hematoxylin and eosin stain, original magnification ×40). (C) A considerable number of melanocytes was scattered in the stroma around the cancer cell nests (hematoxylin and eosin stain, original magnification ×200).

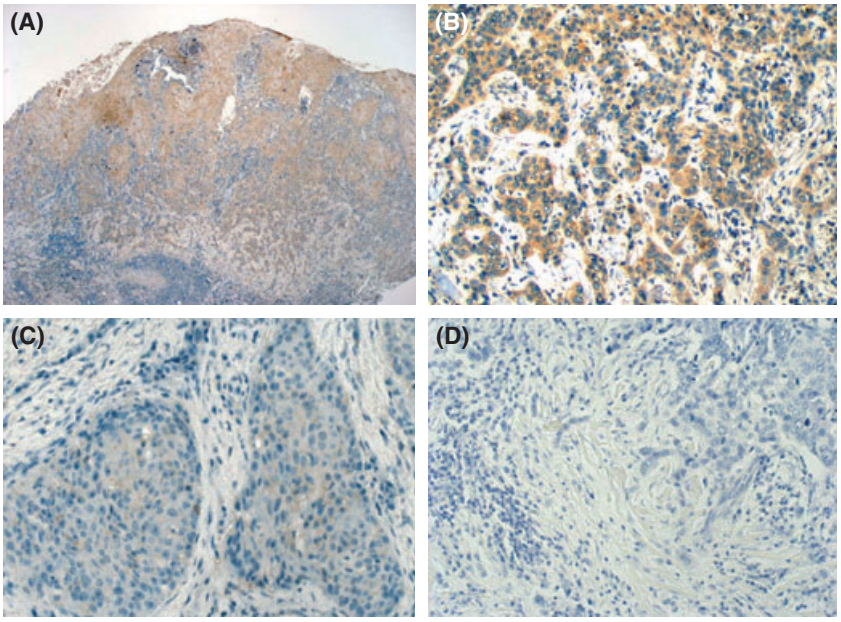

Figure 2 Immunohistochemical localization of stem cell factor (SCF) in the pigmented squamous cell carcinoma (SCC). (A and B) Prominent and homogeneous expression of SCF was observed in the neoplastic keratinocytes of the pigmented SCC (original magnification A: ×40, B: ×200) (C) Only a faint expression of SCF was observed in neoplastic keratinocytes of non-pigmented SCC (original magnification ×200) (D) Negative control (original magnification ×200).

proliferative phase, we performed an immunohistochemical analysis for proliferating cell nuclear antigen (PCNA). The analysis showed that the melanocytes in the present pigmented SCC were noted to be negative for PCNA, indicating that they were not proliferative (Fig. 4).

Comments

Among malignant neoplasms of the oral mucosa, SCC is the most common histopathologic type and usually consists of a pure neoplastic keratinocytes without pigmentation (1). Although it has been stated that

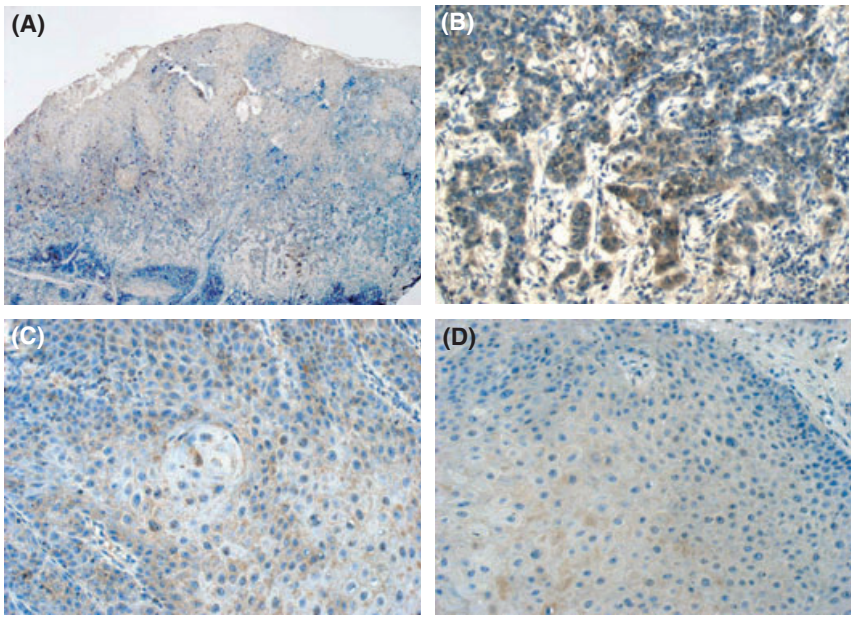

Figure 3 Immunohistochemical localization of endothelin-1 in the pigmented squamous cell carcinoma (SCC). (A and B) Prominent expression of endothelin-1 was noted in the invading neoplastic keratinocytes of the pigmented SCC. (original magnification A: ×40, B: ×200) (C and D) A slight expression of endothelin-1 was noted in some neoplastic keratinocytes of non-pigmented SCCs. (original magnification ×200).

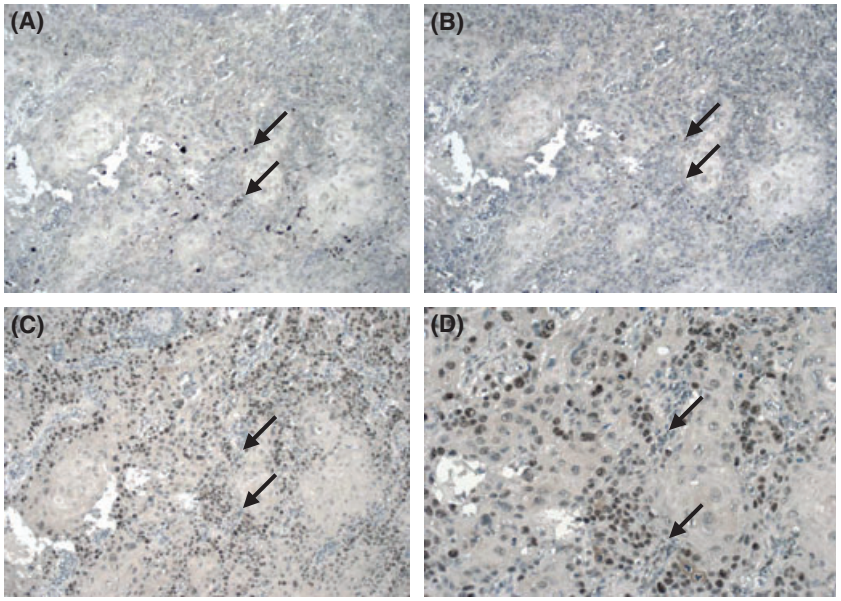

Figure 4 Immunohistochemical detection of proliferating cell nuclear antigen (PCNA) in melanocytes of the pigmented squamous cell carcinoma (SCC) in a single section. (A) Scattered melanocytes (arrows) were observed in the pigmented SCC (hematoxylin stain, original magnification ×100) (B) Melanin bleaching with H_2O_2 quenched the pigment in melanocytes (arrows). (original magnification ×100) (C and D) Immunohistochemical analysis revealed that the PCNA was expressed in neoplastic keratinocytes, but not in melanocytes (arrows). (original magnification C: ×100, D: ×200).

melanocyte colonization is a well-recognized occurrence in a variety of tumors of the epidermis and the hair follicles, it is very rare in oral carcinomas (2, 3) We described here a case of pigmented SCC of the hard palate, and review the clinicopathological characteristics of the eight reported cases – including the present case of pigmented SCC in the oral mucosa (4–8). The mean age of patients was 71.8 years, with a range of 47–81 years. Most of the tumors were not so large, with a mean diameter of 24 mm and a range of 10–30 mm. Simple excision was performed in three of five cases, excision and lymph node dissection in one, and excision and radiation therapy in one. With early diagnosis and treatment or no clinical findings of lymph node metastasis, most patients with ordinary non-pigmented SCCs of the oral mucosa have a favorable prognosis (9).

Pigmented SCCs thus appears to exhibit nearly the same biological behavior as non-pigmented SCCs.

Although melanocytosis (melanosis) or marked pigmentation has been reported in various types of tumor including basal cell carcinoma (10), and breast adenocarcinoma (11), the mechanism of melanocytosis has yet to be elucidated. In contrast, accumulating evidence from *in vitro* experiments has shown that normal keratinocytes produce a variety of growth factors – including SCF, HGF, bFGF, endothelins and NGF – that known to regulate proliferation and/or differentiation of melanocytes (11). It is therefore, reasonable to postulate that neoplastic keratinocytes in pigmented SCC produce and secrete some of these growth factors, leading to the emergence of malanocytosis. In this context, we examined the expression of SCF and endothelin-1 in pigmented SCCs of the present case. SCF is known to stimulate the proliferation of melanoblasts and melanocytes by accelerating the transitions from G0 to G1 and G2 to M phases of the cell cycle. In contrast, endothelin-1 has been reported to stimulate the DNA synthesis and melanogenesis of melanoblasts and melanocytes. Our immunohistochemical examination revealed that neoplastic keratinocytes in pigmented SCC produced much more SCF and endothelin-1 than did non-pigmented SCCs. These results strongly suggest that SCF and/or endothelin-1 secreted by neoplastic keratinocytes in the pigmented SCC stimulate proliferation and/or differentiation of the melanocytes, leading to the emergence of a rare variant of SCC in the oral mucosa. Lan et al. (10) have reported that a pigmented basal cell carcinoma exhibited prominent expression of endothelin-1, while the non-pigmented basal cell carcinoma showed little endothelin-1 expression. They also showed that endothelin-1 secreted by neoplastic keratinocytes induced the melanogenesis but not proliferation. To examine whether the melanocytes in the present case were in the proliferative phase of the cell cycle, we investigated immunohistochemically the expression of PCNA in the melanocytes. This analysis revealed that the melanocytes in the present case were not in the proliferative phase, and strongly suggests that SCF and/or endothelin-1 may stimulate the melanogenesis or migration of melanocytes.

The mechanism underlying the enhanced expression of SCF and endothelin-1 in the neoplastic keratinocytes of the pigmented SCCs is still unclear in this study. However, judging from the fact that the production and secretion of endothelins by epidermal cells are augmented by several inflammatory cytokines such as IL-1α and TNF-α (12, 13), it seems likely that an inflammatory reaction around a tumor may trigger the enhanced expression of SCF and/or endothelin-1. It is also conceivable that melanogenic cytokines other than SCF and endothelin-1 are involved in the pigmentation process of SCC, so further studies are needed to fully elucidate this complex phenomenon. Our results, nevertheless, demonstrate for the first time that at least the enhanced expression of SCF and endothelin-1 on SCC cells is associated with the stimulation of melanocytes, leading to hyperpigmentation in SCCs.

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