Horrifying Basal Cell Carcinoma:
Cytological, Immunohistochemical, and Ultrastructural Findings

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Key Words
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Abstract
Basal cell carcinoma (BCC) is a slow-growing and frequently occurring tumor of the eyelids. Among BCC cases, there is a subtype of aggressive cases called horrifying BCC (HBCC). There are also rare BCC cases that show neuroendocrine differentiation. Here, we describe a case of HBCC with neuroendocrine differentiation. The patient, a 41-year-old woman, presented with abnormal left eye tearing and left cheek pain. On computed tomography imaging, a tumor that extended to the left orbit was detected in the left cheek. On cytological examination of fine-needle aspiration (FNA) samples, the tumor cells were observed as sheet-like clusters and single bare nuclei with a clear background; peripheral palisading was not clearly seen. On examination of the biopsy specimen taken after FNA, the tumor was found to be composed of cancer cell nests with scattered peripheral palisading in the dermis. Immunohistochemically, the tumor cells were positive for cytokeratin (CK) 7 and CD56 and were negative for CK20, synaptophysin, and chromogranin A. Membrane-bound dense-core granules were detected on ultrastructural study. A HBCC case with neuroendocrine differentiation has not been previously reported. The correlation between the presence of neuroendocrine differentiation in HBCC and patient prognosis should be further studied.
Introduction

Basal cell carcinoma (BCC) is the most common malignant periorcular tumor, constituting approximately 90% of surface eyelid malignancies; it is characterized as slow-growing, with low malignant potential [1]. Rarely, cases of so-called horrifying basal cell carcinoma (HBCC) occur, representing a subtype of BCC that displays a destructive behavior [1–5]. BCC is thought to be a tumor derived from hair follicle stem cells [6]. In general, BCC cells do not differentiate to cells with neuroendocrine properties. We report an unusual case of HBCC with neuroendocrine differentiation and invasion of the orbit and cheek.

Case Report

Clinical Findings

A 41-year-old woman with a medical history of allergic conjunctivitis presented to the Kansai Medical University Takii Hospital with a chief complaint of abnormal tearing from the left eye accompanied by left cheek pain. On computed tomography, a mass with unclear borders was found in the left cheek; it invaded the extraocular muscle and subcutaneous fatty tissues, involving the lacrimal gland and the lateral rectus muscle (fig. 1). In addition, the tumor nearly invaded the left ocular sclera. BCC was suspected based on positive cytological examination of fine-needle aspiration (FNA) samples from the orbital cavity. BCC with neuroendocrine differentiation was confirmed by biopsy, which was performed after cytology. Taken together, HBCC with neuroendocrine differentiation was diagnosed. No other lesions were detected by a systemic search, and no specific findings were identified on blood examination. Due to the invasiveness of the tumor, the patient received radiotherapy, and her course has been uneventful as of 10 months after the first diagnosis.

Cytological and Histological Findings

After FNA, the aspiration needle was washed using CytoLyt Solution® (Hologic, Marlborough, Mass., USA). Then, the washed sample was centrifuged at 3,000 rpm for 5 min and processed using a ThinPrep2000® processor (Hologic) according to the manufacturer’s instructions. Cytological examination revealed several cellular clusters, which exhibited poor anisocytosis, loss of polarity, and unclear cell borders in a clear background. Cytologically, isolated bare cells were frequent; the cells were observed to be small in size with scant, insufficient cytoplasm and round to oval nuclei containing fine granular chromatin. Mitosis and single cell necrosis were occasionally seen (fig. 2a). However, peripheral palisading was not noticeable in the cell clusters. Immunocytochemically, the tumor cells were positive for cytokeratin (CK) 7 (fig. 2b) and negative for CK20, CD56, synaptophysin, and chromogranin. Thus, HBCC was suspected both cytologically and clinically. The biopsy sample from the orbit was fixed with 20% neutral buffered formalin and embedded in paraffin; the sample was sectioned at 3 µm and stained with hematoxylin and eosin (HE). Serially cut sections were used for immunohistochemistry. In brief, immunohistochemical examination was performed employing the amino acid polymer method using a Nichirei Histostainer (Nichirei, Tokyo, Japan). After histopathological diagnosis, the tissue block was deparaffinized and used for electron microscopic study. Histologically, tumor cell nests with peripheral palisading were scattered in the dermis. The tumor cells had round to oval nuclei containing fine granular chromatin. Mitotic and single cell necrosis were occasionally seen (fig. 3a). Immunohistochemically, the tumor cells were diffusely positive for CK7 (fig. 3c) and focally positive for CD56 (fig. 3d); they were negative for CK20, synaptophysin, and chromogranin A, and the
Ki-67 labeling index was approximately 70%. As some of the tumor cells from the biopsy specimen selectively stained positive for CD56, the sample was further studied ultrastructurally. Electron microscopically, 200–300 nm-sized membrane-bound dense-core granules were detected in the tumor cell cytoplasm (fig. 3e).

**Discussion**

BCC is the most commonly found periocular malignancy. Although most BCC cases can be cured by local surgical excision, in rare cases BCC may demonstrate more aggressive behavior that can result in orbital and intracranial invasion. These cases are known as so-called HBCC [2–5, 7]. Jackson et al. [2] defined the diagnostic criteria of HBCC as follows: tumor size >3 cm, with the existence of local destruction, recurrence, and metastasis. Our case was in accordance with these criteria. Cases of BCC that exhibit neuroendocrine differentiation are also rare [8–11]. In one report, only two of 53 BCC cases showed neuroendocrine differentiation [10]. Moreover, even when neuroendocrine markers are detected immunohistochemically, the ultrastructural detection of dense-core granules is rather difficult [8, 9]. However, in the present case, neuroendocrine differentiation was confirmed both immunohistochemically and electron microscopically; tumor cells were focally positive for CD56, and membrane-bound dense-core granules were detected from the biopsy specimen on electron microscopy. However, as the CD56-positive cells detected in the biopsy specimen were a minor component, CD56-positive cells were not identified in the cytological specimen. Cytologically, BCC has the following characteristics: (i) variably sized sheets and clusters with peripheral palisading, (ii) round- to oval-shaped nuclei with fine or slightly clumped chromatin, (iii) small nucleoli, (iv) occasional mitotic figures and/or single necrotic cells, (v) scant cytoplasm, (vi) isolated bare nuclei, and (vii) a clear background [12, 13]. In this case, although the presence of peripheral palisading was unclear, the other findings fit these diagnostic criteria. Positive neuroendocrine markers can present a diagnostic pitfall, making it necessary to differentiate BCC from Merkel cell carcinoma, cytologically and histologically [14]. However, Merkel cells show a dot-like pattern of CK20 positivity in the perinuclear cytoplasm [14]. In our case, the tumor cells were negative for CK20.

In summary, cells of the present tumor, which was located in the orbit and invaded the inner cheek, exhibited neuroendocrine differentiation. To the best of our knowledge, this is the first reported case of HBCC with neuroendocrine differentiation diagnosed by FNA cytology and histology. Clinically, the association between HBCC and neuroendocrine differentiation has not yet been evaluated. It may be necessary to compare the prognosis of HBCC with or without neuroendocrine differentiation in future studies.

**Disclosure Statement**

The authors have no potential conflicts of interests with respect to the authorship and/or publication of this article.
References


Fig. 1. Computed tomography imaging. The tumor extended from the left orbit to the cheek.
Fig. 2. Cytological findings based on FNA. a The tumor cells show sheet-like clusters and single bare nuclei. Peripheral palisading was unclear. The nucleus-to-cytoplasm ratio was high, an increased amount of fine granular chromatin was observed, and mitotic figures (red arrowhead) were occasionally seen (Papanicolaou staining, ×400). b Tumor cells were diffusely positive for CK7 (immunocytochemical staining 7, ×400).
Fig. 3. Histological findings from the biopsy specimen. a, b Tumor cell nests were scattered in the dermis, in which peripheral palisading was seen (HE, a ×100, b ×400). c The tumor cells were diffusely positive for CK7 (immunohistochemical staining, ×400), and d partially positive for CD56 (immunohistochemical staining, ×400). e Electron microscopic findings from the biopsy specimen. Membrane-bound dense-core granules were seen in the cytoplasm (bar: 500 nm).