Undifferentiated sinonasal malignant melanoma: A case report

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Abstract. Undifferentiated sinonasal malignant melanoma (MM) is a rare type of tumor, which can be easily misdiagnosed. The present study reports a 41-year-old male patient who presented with a 4-day history of epistaxis. Clinical examination and radiological imaging lead to the detection of a mass in the right sinonasal region. Histopathological examination revealed that the mass was composed of malignant epithelioid cells arranged in nests and sheets. These cells displayed a hemangiopericytoma-like pattern with antler-like branching vessels. Immunohistochemical staining revealed that the tumor cells exhibited negative expression of melanocytic markers. This increased the difficulty of distinguishing undifferentiated MM from other malignant tumors located in the sinonasal area, particularly undifferentiated nasopharyngeal carcinoma. The diagnosis of undifferentiated MM was determined by ultrastructures, including the mature melanosomes and premelanosomes, in tumor cells by transmission electron microscopy. The present study suggests that the analysis of cancer stem cell marker and vasculogenic mimicry may be an important auxiliary tool for the diagnosis of MM.

Introduction

Malignant melanoma (MM) is a type of tumor arising from melanocytes, which originate from neural crest cells (1). The common primary sites of MM are the skin and mucosal surfaces (2). MM is associated with a very high mortality rate and an extremely poor prognosis (3). Therefore, the accurate diagnosis of MM is essential for providing appropriate and timely treatment. Sinonasal MM accounts for <1% of all melanomas and <5% of all sinonasal tract neoplasms (4). Patients with sinonasal MM often display non-specific symptoms, including nasal obstruction or epistaxis, causing clinical misdiagnosis (1). Based on melanin pigmentation, MM can be histopathologically categorized into melanotic and amelanotic subtypes (1,5). It is difficult to diagnose amelanotic MM in undifferentiated tumor cells as they exhibit negative expression of all melanocytic markers (6). The present study reports a case of undifferentiated sinonasal MM, which mimics a poorly differentiated carcinoma with aberrant expression of epithelial markers. The ultrastructural characteristics of the tumor cells and the tumor phenotypes, including the proportion of cancer stem cells and vaculogenic mimicry (VM), were determined.

Case report

Written informed consent was acquired from the patient discussed in the present report, and the following study was ethically approved by the Ethics Committee of the Anhui Provincial Hospital (Anhui, China). A 41-year-old male patient was admitted to Anhui Provincial Hospital (Hefei, China) in July 2016. The patient reported a month of congestion of the right nasal passage with no rhinorrhea or bleeding until the 4 days prior to presentation, in which progressive epistaxis was experienced. Magnetic resonance imaging (MRI) revealed a lesion in the right nasal cavity and ethmoid sinus (Fig. 1A and B). Nasal endoscopic examination indicated a red mass occupying the right side of the nasal cavity and the middle meatus. The mass had invaded the middle turbinate, nasal septum and sieve plate, and bone destruction had occurred. Complete excision of the primary lesion with at least 1.5 cm of normal tissue was performed, and a sentinel lymph node biopsy achieved a negative result. The otolaryngologist identified the surface of the mass to be rough, brittle and to bleed easily when palpated. The patient exhibited stage II disease at diagnosis, and radiotherapy was indicated.

The specimen was examined by two pathologists. The excised tissue was fixed in 10% neutral-buffered formalin for 10 h at room temperature, then tissue samples were dehydrated in an ethanol/xylene series, embedded using fresh paraffin wax and maintained at 55-60°C. Tissue samples were serially sectioned (4-μm thick); 4 underwent hematoxylin and eosin (H&E) staining and 23 underwent immunohistochemical staining. Immunohistochemistry

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was performed using the ChemMate Envision kit including secondary antibody (cat. no., DAKOK500711; Dako; Agilent Technologies, Inc., Santa Clara, CA, USA), according to the manufacturer's protocol (7). Sections were deparaffinized in xylene and dehydrated with descending alcohol series. Endogenous peroxidase was blocked with 0.1% hydrogen peroxide-methanol for 30 min at room temperature. Sections were washed once with PBS, and then microwaved for 15 min in 0.05 mol Tris buffer (pH 9.0) for antigen retrieval, followed by washing three times with PBS, and then incubated with primary antibodies at 4°C overnight. Following washing three times with PBS, they were incubated with horseradish peroxidase-conjugated dextran polymer reagent (dilution, 1:2,000; cat. no., sc-2004; Santa Cruz Biotechnology, Inc.) for 1 h at room temperature. The sections were incubated with the following 23 primary antibodies: Human melanoma black-45 (mouse monoclonal; cat. no., MAB-0098), Melan-A (mouse monoclonal; cat. no., MAB-0275), smooth muscle actin (SMA; mouse monoclonal; cat. no., Kit-0006), marker of proliferation Ki67 (mouse monoclonal; cat. no., Kit-0005), synaptophysin (Syn; rabbit monoclonal; cat. no., Kit-0022), cluster of differentiation 31 (CD31; mouse monoclonal; cat. no., MAB-0031), friend leukemia integration 1 transcription factor (Fli-1; mouse monoclonal; cat. no., MAB-0649), cluster of differentiation 21 (CD21; rabbit monoclonal; cat. no., RMA-0647), factor VIII (rabbit polyclonal; cat no., RAB-0070; all from Fuzhou Maixin Biotechnology Development Co., Ltd., Fuzhou, China). The following antibodies were purchased from ZSGB-BIO (Beijing, China): pan-cytokeratin (mouse monoclonal; cat. no., ZM-0069), vimentin (mouse monoclonal; cat. no., ZM-0260), cytokeratin 5 (rabbit monoclonal; cat. no., ZA-0518), CD34 (mouse monoclonal; cat. no., ZM-0046), epithelial membrane antigen (EMA; mouse monoclonal; cat. no., ZM-0095), S100 (rabbit polyclonal; cat. no., ZA-0225), cluster of differentiation 45 (CD45; mouse monoclonal; cat. no., ZM-0183), p40 (mouse monoclonal; cat. no., ZM-0472), sex determining region Y-box 10 (SOX10; rabbit monoclonal; cat. no., ZA-0624). Primary antibodies (volume, 100 μl) were provided as ready-to-use products and did not require further dilution. The cancer stem cells markers used include the following primary antibodies: Oct4 (rabbit polyclonal; dilution, 1:200; cat. no., 2750), sex determining region Y-box 2 (SOX2; rabbit monoclonal; dilution, 1:100; cat. no., 3579), Nanog (rabbit monoclonal; dilution, 1:800; cat. no., 4903; all from Cell Signaling Technology, Inc., Danvers, MA, USA), cluster of differentiation 133 (CD133; rabbit polyclonal; dilution, 1:100; cat. no., sc-30220; Santa Cruz Biotechnology, Inc., Dallas, TX, USA) and Nestin (mouse monoclonal; dilution, 1:200; cat. no., MAB-0566; Fuzhou Maixin Biotechnology).

Gross pathological examination resulted in the description of a grey irregular specimen of 5x5x1 cm^3 and moderate hardness. Histopathologically, the mass was composed of malignant epithelioid cells arranged in nests and sheets (Fig. 2A). The overall cellularity was moderate to high with evident atypia. The tumor cells displayed a hemangiopericytoma-like pattern accompanied by antler-like branching vessels. The tumor stroma was focally desmoplastic and necrosis was not evident. Under a light microscope at x400, magnification (Leica 2500 microscope; Leica Microsystems, Inc., Buffalo Grove, IL, USA), the tumor cells demonstrated relatively uniform hyperchromatic, rounded nuclei with prominent nucleoli, and mitotic activity was conspicuous. The cytoplasm of the tumor was lightly eosinophilic to amphophilic, containing numerous vacuoles but exhibiting no pigment deposition (Fig. 2B).

Immunohistochemical analyses demonstrated that the tumor cells did not express melanocytic markers HMB-45, Melan-A, S100 or SOX10 (Fig. 3A). However, the tumor cells did express vimentin (Fig. 3B), EMA (Fig. 3C) and Fli-1 (Fig. 3D). Pan-cytokeratin expression demonstrated scattered expression among tumor cells (Fig. 3E). The tumor cells did not express cytokeratin 5, CD31, CD34, Syn, CD45, CD21, SMA, Factor VIII, p63 or p40. A total of 80% of the tumor cells were positive for Ki-67 staining (Fig. 3F). Ultrastructural analysis by transmission electron microscopy was performed to determine the malignancy type. The results demonstrated that mature melanosomes and premelanosomes existed in tumor cells (Fig. 4), supporting the diagnosis of MM.

In order to further understand the nature of undifferentiated MM, cancer stem cell marker expression was also analyzed, including that of CD133, SOX2, OCT4 and Nanog. The tumor cells expressed CD133 strongly and diffusely (Fig. 5A). SOX2 was expressed focally around the vessels (Fig. 5B), while nestin expression was negative for tumor cells, but positive for vascular mural cells (Fig. 5C). The tumor cells exhibited negative expression of OCT4 and Nanog. In order to investigate VM, CD34 and periodic acid-Schiff (PAS; B24200-250; Thermo Fisher Scientific, Inc., Waltham, MA, USA) double-staining was used to distinguish microvessels from VM. Sections were exposed to sodium periodate for 10 min following immunohistochemical staining of CD34 at room temperature, then rinsed with distilled water for 5 min, followed by incubation with 0.1% Periodic acid-Schiff for 15 min at room temperature. All sections were counterstained with Mayer's hematoxylin for 2 min at room temperature, dehydrated with descending alcohol series, and mounted. VM was characterized as a channel positive for PAS without exhibiting positive CD34 staining of the endothelium (Fig. 5D).

Discussion

Primary MM of the sinonasal mucosa is a rare disease, with an equal incidence in males and females. It often occurs at 60-70 years of age, but rarely prior to 40 years of age (8). Obstruction or epistaxis are the most common early symptoms of sinonasal MM (1). Sinonasal MM has a high rate of local recurrence and distant metastasis (1,3). According to previous literature, the 5-year survival rate of patients is 44-88.7% (9). One previous study revealed that the majority of patients with sinonasal MM exhibiting stage I or II disease demonstrated no evidence of recurrence after a follow-up period of 13.9 years. However, all patients presenting with a stage III diagnosis had succumbed to the disease during the follow-up period (10). The survival rate was associated with tumor location, tumor spread, tumor thickness, lymph node and distant metastases (9,10). Therefore, the early and accurate diagnosis of sinonasal MM is essential for effective treatment of the disease.

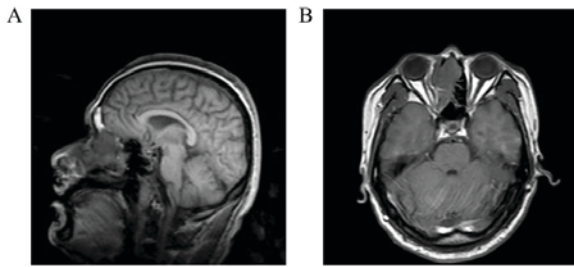Histopathologically, melanoma is categorized into melanotic and amelanotic types according to melanin

Figure 1. Preoperative magnetic resonance imaging. (A) Sagittal view and (B) axial view, demonstrating a mass occupying the right side of the nasal cavity, extending forward toward the right ethmoid sinus with erosion of adjacent bone tissues.
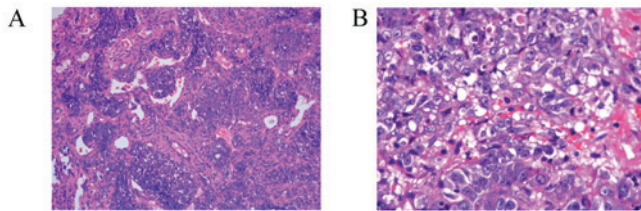

Figure 2. Histopathological analysis of the tumor tissue. (A) H&E staining revealed that the tumor exhibited a nests and sheets arrangement, and displayed a hemangiopericytoma-like pattern with antler-like branching vessels (magnification, x100). (B) Higher magnification (x400) revealed that the tumor was composed of uniform epithelioid cells, with hyperchromatic, rounded or ovoid nuclei with prominent nucleoli and high mitotic activity. H&E, hematoxylin and eosin.
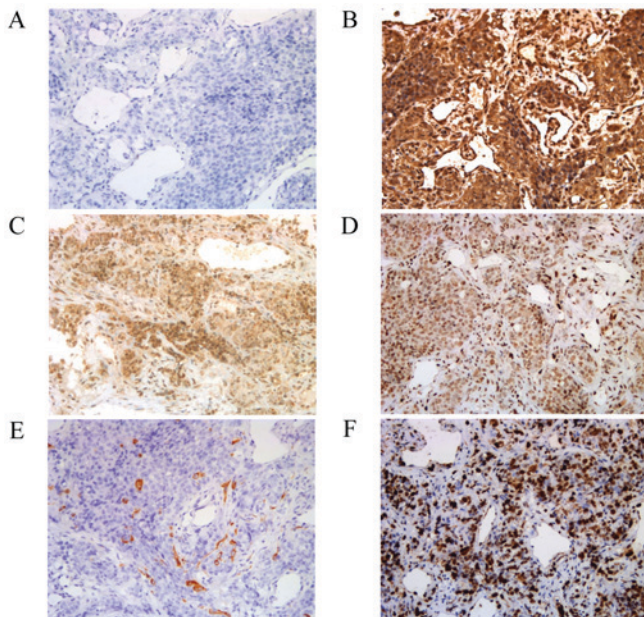

Figure 3. Immunohistochemical analysis of undifferentiated malignant melanoma. (A) The tumor cells did not express human melanoma black-45. (B) The tumor cells were positive for vimentin expression. (C) The tumor cells were positive for epithelial membrane antigen expression. (D) The tumor cells were positive for friend leukemia integration 1 transcription factor nucleic expression. (E) The tumor cells exhibited scattered expression of pan-cytokeratin. (F) Ki-67 expression was positive in the nuclei of 80% tumor cells. Magnification, x200.
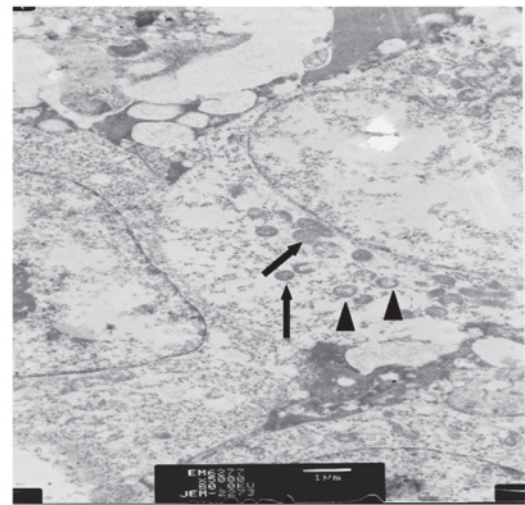

Figure 4. The ultrastructure of the undifferentiated malignant melanoma. Transmission electron microscopy detected a small number of mature melanosomes, indicated by black arrows, and premelanosomes, indicated by black arrowheads, within the tumor cells (scale bar: 1 μm).
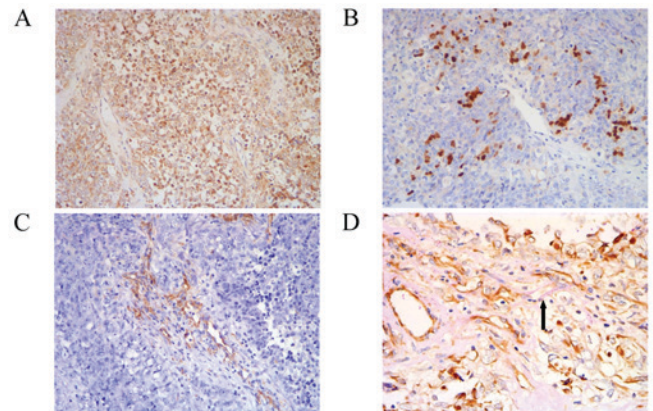

Figure 5. Cancer stem cells markers and vasculogenic mimicry detection in undifferentiated malignant melanoma. (A) The tumor cells were positive for CD133 expression (magnification, x200). (B) The tumor cells exhibited scattered expression of sex determining region Y-box 2 (magnification, x200). (C) The tumor cells did not express Nestin, but did exhibit vascular mural cells (magnification, x200). (D) CD34/PAS double staining revealed vasculogenic mimicry positive for PAS staining (purple-red) and negative for CD34 (black arrow). The microvessels were positive for both CD34 and PAS staining (magnification, x400). CD133, cluster of differentiation 133; CD34, cluster of differentiation 34; PAS, periodic acid-Schiff.

mixed-cell (12). In a number of cases, the melanoma cells may be undifferentiated with no expression of melanocytic markers, resulting in difficulty regarding its diagnosis (6,13).

In the present case report, the tumor cells exhibited negative expression of melanocytic markers, including HMB-45, Melan-A, S100 and SOX10. However, the tumor cells exhibited positive expression of epithelial markers, including EMA and pan-cytokeratin. These characteristics mimic those of undifferentiated nasopharyngeal carcinomas that occur commonly in sinonasal regions (14). However, characteristically, undifferentiated nasopharyngeal carcinomas exhibit cytokeratin 5, p63 and p40 expression (15), which was not exhibited by the tissue analyzed in the present study. On the other hand, expression of the endothelial cell

pigmentation (11). MM tumors are composed of a variety of cell types: Epithelioid, spindled, clear-cell, plasmacytoid and

marker, Fli-1, was observed, which raised the possibility of epithelioid angiosarcoma, characterized by Fli-1, CD31, CD34 and factor VIII expression (16). However, a previous study demonstrated that Fli-1 may be strongly expressed in MM, and that Fli-1 expression is associated with tumor cell proliferation rate and other aggressive behaviors (17). The immunohistochemical panel of the present study excluded other possible tumor types of the sinonasal region, including sinonasal neuroendocrine carcinoma, paraganglioma, olfactory neuroblastoma, pituitary adenoma, tumors of the Ewing family and follicular dendritic cell sarcoma. Sinonasal neuroendocrine carcinoma, paraganglioma, olfactory neuroblastoma, pituitary adenoma and tumors of the Ewing family which usually express Syn; however this was not expressed in the present case study. Follicular dendritic cell sarcoma demonstrated diffuse staining with CD21, while MM was negative for CD21. The results of transmission electron microscopy analysis revealed the presence of mature melanosomes and premelanosomes in the cytoplasm of the tumor cells, which are key ultrastructures of MM cells (18).

SOX2, CD133 and Nestin have been reported as cancer stem cell markers in melanoma (19-21). In the present study, the tumor cells of undifferentiated MM expressed CD133 and SOX2. Nestin was not expressed in tumor cells but was expressed in vascular mural cells. It is speculated that cancer stem cells may transdifferentiate into pericytes and participate in blood vessel remodeling (22,23). Tumor cell vasculogenic mimicry (VM) refers to the plasticity of cancer cells forming *de novo* vascular networks, and is associated with malignant phenotypes and a poor prognosis (24,25). Cancer stem cells may contribute to the formation of VM (26). VM was originally identified in malignant melanoma, and was subsequently demonstrated to be an important morphological feature of MM (27). In the present study, VM is detectable in undifferentiated MM due to stemness, which may be a useful tool for MM diagnosis.

The present study presents a rare case of undifferentiated sinonasal MM. Given that an accurate diagnosis of this type of tumor is challenging, it is demonstrated that the combination of a panel of conventional immunohistochemical markers, ultrastructure identification by transmission electron microscopy and the VM detection may be used to validate the diagnosis of MM, and to exclude other malignancies of the sinonasal area.

Acknowledgements

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

JD and LLH contributed to the design of the research, drafting the manuscript, gave final approval of the version to be published, and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. AX, ALZ, XK, MD, WH and ZLG performed the experiments. WZ, SBS and HL analyzed the data. JC and QS made substantial contributions to conception and acquisition of data. LLX made contributions to interpretation of data. HBW contributed to the design of the research, and revised and finalized the article. All authors read and approved the final manuscript.

Ethics approval and consent to participate

This study was ethically approved by the Ethics Committee of the Anhui Provincial Hospital (Anhui, China). Informed consent was obtained from the study participant.

Consent for publication

All patients provided consent for the publication of their data

Competing interests

The authors declare that they have no competing interests.

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