Histological, immunological and molecular features of a nasal mucosa primary melanoma associated with nasal melanosis

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Abstract: Nasal mucosa melanoma is a rare entity that may occur together with nasal melanosis. The histological and immunological features and loss of heterozygosity analysis of such lesions have not been reported to date. In the study presented here short-term cell cultures were established from the patient’s melanoma and subsequent relapses. Histology, immunohistochemistry, reverse transcription-polymerase chain reaction enzyme-linked immunosorbent assay, human leukocyte antigen analysis, microdissection with subsequent polymerase chain reaction for analysis of loss of heterozygosity were used to characterize the tumour and other cells. Melanoma of the nasal cavity was found, with a surrounding proliferation of atypical melanocytes corresponding to nasal melanosis. Immunoreactivity was found for S-100, gp100, tyrosinase and MelanA protein. Loss of heterozygosity for a p16-flanking marker was found in the tumour and the melanotic cells. Short-term cell cultures expressed tyrosinase and MUC18 at the mRNA level and intercellular adhesion molecule-1 (ICAM-1) and interleukin-12 receptor at the protein level. This is the first time short-term cell cultures have been established and analysed from such a tumour. Melanoma-associated antigens were identified within the tumour. The melanoma and the melanotic cells showed loss of heterozygosity for the p16 gene, which is implicated in melanoma development. This points to a common origin in tumorigenesis. Pathways of tumour escape, such as expression of CD54 and interleukin-10, were observed. The clinical, immunological and molecular features suggest that nasal melanosis should be followed closely.

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Histological, immunological and molecular features of a nasal mucosa primary melanoma associated with nasal melanosis


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Nasal mucosa melanoma is a rare entity that may occur together with nasal melanosis. The histological and immunological features and loss of heterozygosity analysis of such lesions have not been reported to date. In the study presented here short-term cell cultures were established from the patient’s melanoma and subsequent relapses. Histology, immunohistochemistry, reverse transcription-polymerase chain reaction enzyme-linked immunosorbent assay, human leukocyte antigen analysis, microdissection with subsequent polymerase chain reaction for analysis of loss of heterozygosity were used to characterize the tumour and other cells. Melanoma of the nasal cavity was found, with a surrounding proliferation of atypical melanocytes corresponding to nasal melanosis. Immunoreactivity was found for S-100, gp100, tyrosinase and MelanA protein. Loss of heterozygosity for a p16-flanking marker was found in the tumour and the melanotic cells. Short-term cell cultures expressed tyrosinase and MUC18 at the mRNA level and intercellular adhesion molecule-1 (ICAM-1) and interleukin-12 receptor at the protein level. This is the first time short-term cell cultures have been established and analysed from such a tumour. Melanoma-associated antigens were identified within the tumour. The melanoma and the melanotic cells showed loss of heterozygosity for the p16 gene, which is implicated in melanoma development. This points to a common origin in tumorigenesis. Pathways of tumour escape, such as expression of CD54 and interleukin-10, were observed. The clinical, immunological and molecular features suggest that nasal melanosis should be followed closely. © 2002 Lippincott Williams & Wilkins

Key words: melanoma, melanosis, mucosa, non-cutaneous melanoma, sinonasal melanoma

Introduction

Mucosal melanosis is a prominent hyperpigmentation of the basal layer that is accentuated at the tip of the rete ridges.1 Malignant melanoma of the nasal cavity or paranasal sinus is a rare disorder.2 Mucosal melanosis of the nasal or paranasal mucosa in association with melanoma is even more uncommon. A relationship between nasal melanosis and increased incidence of nasal and paranasal melanoma has been suggested in persons of African descent3 and has been documented in one patient.4 We report a novel case of nasal melanoma in a Caucasian patient with mucosal melanosis that was analysed at the molecular level.

Case report

A 39-year-old white male presented to the Departments of Head and Neck Surgery and Dermatology at our institution in June 1994. He had a 4-year history of obstructed nose breathing, and complained of a decreased sense of smell and occasional epistaxis. In April 1994 he had noticed a nodule growing in his left nostril. At the time of presentation, an endonasal endoscopic procedure with anterior ethmoidectomy and conchotomy was performed. The histological examination revealed malignant melanoma with mucosal polypsis of the paranasal sinuses.

Imaging studies, including positron emission tomography (PET),5 were performed for staging purposes: remaining tumour, with no metastases, was detected (pT3N0M0). During an endonasal endoscopic revision of the residual tumour in the left maxillary and anterior ethmoid sinuses, extensive mucosal melanosis was discovered within the left nasal cavity (see Figure 1).
In November 1994 a ‘second-look’ procedure was performed, and a local recurrence and two foci of melanosis were removed. Adjuvant percutaneous radiation therapy (total dose of 54 Gy) was administered to the nose, ethmoid cavity and cervical lymph nodes. In March 1995 a third local recurrence was observed and was again endoscopically removed by the Ear, Nose and Throat Department.

Interferon-α2b and interleukin-2 were given as adjuvant therapy from April 1995 to March 1996. In September 1995 a fourth local recurrence of the malignant melanoma was treated by revision of the endonasal medial maxillectomy and a subtotal mucosa resection of the nasal septum on the left side. In June 1996 suspicious alterations in lymph nodes were noted on computed tomography (CT) and PET

Figure 1. (a) Mucosal melanosis of the left nasal septum as seen on endoscopy. Primary melanoma showing the melanosis at periphery stained with hematoxylin-eosin. (b) and stained for S100. (c) gp100. (d) Tyrosinase. (e) and MelanA/MART-1. (f) (original magnification 100×).
scan. A left-sided modified radical neck dissection was performed in July 1996. Five of the 34 lymph nodes removed were positive for metastasis. In November 1996 imaging studies showed local and regional recurrence. In December 1996 a cervical lymphadenectomy yielded a positive lymph node and the original tumour site was surgically revised. An additional cervical lymphadenectomy was performed in February 1997, yielding another positive lymph node.

As a part of a clinical study, the patient received dendritic cell vaccination with melanoma-associated antigen peptides. Six injections into the inguinal lymph nodes with gp100-, tyrosinase- and MelanA/MART-1-pulsed dendritic cells were performed between February 1997 and April 1997. The nasal tumour mass decreased on imaging studies, but metastases in the right upper pulmonary lobe, the lingula and the pancreas were observed. In June 1997 another lymphadenectomy, a partial resection of the nasal septum, and extended local revision and resection of a satellite metastasis of the left cheek were performed.

In June 1997 a recombinant canary-pox virus transduced with granulocyte/macrophage colony stimulating factor (GMCSF) was applied to a subcutaneous metastasis on the left neck as part of a phase I clinical trial. Imaging studies in July 1997 showed progression of pulmonary and abdominal metastases and new metastases to the liver and adrenal glands. Palliative chemoimmunotherapy using dacarbazine, cisplatin, interferon-α and interleukin-12 was initiated in July 1997. The patient received six treatment cycles. Dosage was adjusted to minimize side-effects, which included nausea, emesis, diarrhoea, fatigue, erosive oral ulcerations and acute renal insufficiency with oliguria. The dosage of interferon-α was reduced due to recurring leukopenia. After the last cycle of chemoimmunotherapy in January 1998, imaging studies demonstrated further progressive disease in the maxillary sinus, chest and abdomen, but no new lesions were noted.

In March 1998 the patient developed progressive visual loss and headache. Multiple cerebral metastases, as well as progression of the previously known metastases, were detected on imaging studies. Additionally, the patient suffered a pathological fracture of the right upper arm, which was treated surgically. Systemic steroids were given, and radiotherapy with a total dose of 20 Gy was administered to the brain. The cerebral symptoms improved slightly. In June 1998 the patient succumbed to his disease.

**Methods**

Formalin-fixed paraffin-embedded tissue was used for routine histology and for immunohistochemistry using monoclonal antibodies and the alkaline phosphatase–anti-alkaline phosphatase method. Total RNA from short-term melanoma cell cultures established from two lymph node metastases were analysed by reverse transcription-polymerase chain reaction enzyme-linked immunosorbent assay (RT-PCR ELISA) for tyrosinase, MUC18, MAGE-3 and MelanA/MART-1 mRNA expression.

The human leukocyte antigen (HLA) status of peripheral blood lymphocytes and of melanoma cells from metastases was determined using a standard microcytotoxicity assay (Dynal, Milan, La Roche, Switzerland) as described elsewhere. Short-term melanoma cell cultures were analysed for HLA class I, HLA class II molecules, intercellular adhesion molecule-1 (ICAM-1), NCAM and interleukin-12 receptor using flow cytometry.

Between five and 50 atypical melanocytic cells of the respiratory epithelium, corresponding to the area of mucosal melanosis, malignant melanoma tumour cells and normal epidermal cells were microdissected under light microscope visualization (magnification ×200) using a 30 gauge needle from two slides for loss of heterozygosity (LOH) analysis. Procured cells were immediately suspended in 1 g/l proteinase K, pH 8.0, and incubated for 2 days at 37°C. LOH analysis was carried out by PCR amplification of microsatellite polymorphisms using two polymorphic DNA markers at chromosome 9p21, IFNA and D9S171, which are flanking markers of the p16 gene (Research Genetics, Huntsville, Alabama, USA), as described elsewhere.

**Results**

Gross and light microscopic features

The primary tumour measured 1.5 cm in diameter on an area of brown-black pigmented mucosa. The surface was slightly rugged, glistening and dark black, with occasional traces of blood. Microscopically, we found sheets and small nodules of tumour cells in the lamina propria, with ulceration of the covering respiratory epithelium. The tumour was composed of large atypical cells with abundant cytoplasm, large hyperchromatic nuclei and prominent nucleoli. Brown melanin pigment was present within the cytoplasm of these cells. Mitotic figures were numerous and some were atypical. Focally,
neoplastic pleomorphic cells were more spindle-shaped. Stains for S-100 protein, tyrosinase, MelanA and gp100 were strongly positive. Alongside the malignant melanoma we found areas showing proliferation of atypical melanocytes in the respiratory epithelium. These cells were strongly immunopositive for the same markers as the malignant melanoma.

In subsequent biopsies of local relapses and metastases, melanoma-associated antigens remained detectable on immunohistochemistry. Increased numbers of antigen-specific CD8+ T cells were isolated from peripheral blood mononuclear cells following dendritic cell vaccine (data not shown).

LOH

Microdissected atypical melanocytic cells of the respiratory epithelium corresponding to the area of mucosal melanosis showed loss of the lower allele using the polymorphic marker D9S171, whereas the malignant melanoma tumour cells showed loss of both alleles of this flanking marker for the p16 gene (Figure 2). The polymorphic marker IFNA, which is a flanking marker of the p16 gene, was used in the same PCR reaction and showed homozygosity.

mRNA detection by RT-PCR ELISA

Short-term melanoma cell cultures established from two lymph node metastases showed mRNA expression of tyrosinase and MUC18, and no mRNA expression of MAGE-3 or MelanA/MART-1 (data not shown). No expression of FasLigand mRNA was found (data not shown).

HLA status

The HLA status of peripheral blood lymphocytes and melanoma cells from metastases was determined to be HLA-A2, -A3, -B7, -B51 using a standard microcytotoxicity assay. No alteration in HLA status was detected. Short-term melanoma cell cultures from metastases showed constitutive expression of HLA class I and II molecules on flow cytometry, which were upregulated after stimulation with interferon-α and interferon-γ.

Adhesion molecules and cytokine receptors

Short-term melanoma cell cultures from metastases showed constitutive expression of ICAM-1 (CD54), which was upregulated after interferon stimulation. No constitutive expression for NCAM (CD56) was found, but expression was seen after interferon stimulation. Interleukin-12 receptor was detected on unstimulated melanoma cells.

Discussion

Mucosal melanoma represents only 1.3% of all reported melanomas and shows a higher incidence in African-American compared with white non-Hispanic ethnicity. Melanocytes, which can be found in the nasal cavity, probably develop into melanoma. Melanosis of the nasal cavity with melanoma is exceedingly rare, particularly in Caucasians.

The prognosis of patients with intranasal melanoma is poor. Five-year survival rates range from 20 to 60% in historical data; in the most recent study by Chang et al., the 5-year survival for mucosal melanoma is 25%, with tumours in the head and neck region faring slightly better at 31.7%. The diagnosis is usually established at an advanced stage of the disease due to the late development of symptoms, thus delaying treatment. Metastasis can occur both haematogeneously and lymphatically.

In this study it was possible for the first time to cultivate melanocytic tumour cells from a nasal mucosa melanoma. The cells presented no HLA changes compared with peripheral blood mononuclear cells and reacted with normal HLA class I
and II upregulation to interferon-α and γ stimulation. The cells expressed the melanocytic markers S-100, gp100, tyrosinase and MelanA on immunohistochemistry. Based on our investigations, the tumour cells seemed to present no features inhibiting their recognition by cytotoxic T cells. Therefore dendritic cell vaccination was undertaken. Continued melanoma-associated antigen expression (tyrosinase, MelanA/MART-1, TRP-1 and gp100) on the primary tumour and metastases was found both in biopsies and in short-term tumour cell cultures. Dendritic cell vaccination resulted in an increased frequency of cytotoxic CD8+ lymphocytes recognizing melanocytic antigens in the peripheral blood.

Nevertheless, the metastases progressed. Several mechanisms of tumour escape can be recognized in this patient. Constitutive expression of ICAM-1 (CD54), as seen in this patient’s melanoma cell cultures, might facilitate tumour escape by the release of soluble ICAM-1, which interferes with the attachment of cytotoxic T cells. Increased interleukin-12 receptors on the patient’s tumour cells can mediate interleukin-12-induced upregulation of HLA class I, HLA class II and ICAM-1 molecules. However, with HLA class I molecules present on melanoma cells, tumour escape could also be due to deficient antigen processing machinery or to interleukin-10 secretion by melanoma cells.

In the LOH analysis of our patient’s melanotic and tumour cells using the polymorphic marker D9S171, which is a flanking marker for the p16 gene, we found LOH in the area of melanosis and complete loss of both alleles within the tumour. Lacking knowledge about our patient’s nasal mucosa before the diagnosis of melanoma was made, we have no data on the chronological order of melanosis and melanoma development in this case. The occurrence of LOH as an early event in tumorigenesis, however, points to a common origin for both lesions. The repeated local recurrence of melanoma in the area of melanosis supports this concept. The clinical observation of an increased incidence of mucosal melanosis and mucosal melanoma in people of African descent is also suggestive of nasal melanosis as a premalignant condition. Similar associations have been described in ocular melanosis, which some authors consider to be melanoma in situ. Nasal melanosis should therefore be closely followed in affected individuals.

References

18. Köpp H, Köpp R, Maier W, Freudenberg N. Rezidi-


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