Malignant melanoma of the nasal cavity: a case report with examination of *KIT* and p*latelet derived growth factor receptor-α (PDGFRA)*

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Abstract

Although several clinicopathological studies of malignant melanoma of the nasal cavity have been reported, there are no studies of the expression and gene mutation of *KIT* and *platelet derived growth factor receptor-α* (*PDGFRA*) in melanoma of the nasal cavity. A 92-year-old Japanese woman consulted to our hospital because of right nasal obstruction and epistaxis. Physical examination and imaging modalities showed a tumor of the right nasal cavity. A biopsy was taken, and it showed malignant epithelioid cells with melanin deposition. Immunohistochemically, the tumor was positive for S100 protein, HMB45, p53, Ki-67 (labeling=20%), *KIT* and *PDGFRA*. The tumor was negative for cytokeratins (AE1/3 and CAM5.2). A genetic analysis using PCR-direct sequencing revealed no mutation of *KIT* gene (exons 9, 11, 13, and 17) or the *PDGFRA* gene (exons 12 and 18). The pathological diagnosis was primary malignant melanoma of the nasal cavity. The tumor was reduced in size by local resection and chemotherapy (Darthmose regimen: dacarbazine, carmustine, cisplatine, and tamoxifen), and the patient is now alive and free from metastasis 9 months after the first manifestation. In conclusion, the author reported a case of melanoma of the nasal cavity expressing KIT and *PDGFRA* without gene mutations of *KIT* and *PDGFRA*.

Introduction

Malignant melanoma is a highly malignant tumor, and *NRAS* and *BRAF* mutations are mainly involved in the pathogenesis of melanoma.[1,2] *KIT* gene, mapped to 4q12, encodes an oncogenic transmembranous receptor tyrosine kinase, *KIT*, whose ligand is stem cell factor.[3] The *platelet derived growth factor receptor-α* (*PDGFRA*) gene, also mapped to 4q12, also encodes an oncogenic transmembranous receptor tyrosine kinase, *PDGFRA*.[3] The *KIT* gene plays an important role in the melanocyte migration, development, differentiation and tumorigenesis.[4] Previous studies have shown that activating mutations of the *KIT* gene may lead to tumorigenesis of cutaneous melanoma.[1] Since *KIT* and *PDGFRA* genes are mapped to 4q12, it is anticipated that *PDGFRA* gene mutations are involved in the tumorigenesis of melanoma, as in the case of gastrointestinal stromal tumors.[3] However, *PDGFRA* gene mutations in melanoma have rarely been examined.[5-8] In addition, *PDGFRA* protein expression has rarely been analyzed in melanoma. These studies have been performed in Caucasians, and only two reports by Ashida *et al.*[6] and ours[7] are available in Mongoloids, including Japanese, in which malignant melanoma is much more uncommon than in Caucasians. Ashida *et al.*[6] reported that KIT protein expression was 48% in Japanese cutaneous melanoma and that *KIT* mutation was 16% in Japanese cutaneous melanoma. Our previous study[7] has shown that *KIT* and *PDGFRA* expression in cutaneous melanoma was present in 92% and 100%, respectively, and that mutations of *KIT* and *PDGFRA* were recognized in 8% and 0%, respectively, in cutaneous melanoma.

Although several clinicopathological studies on melanoma of the nasal cavity have been performed[9,10] there have been no studies of *KIT* and *PDGFRA* in melanoma of the nasal cavity. In the present study, the author investigated the protein expression and gene mutation status of *KIT* and *PDGFRA* in a case of nasal melanoma of a Japanese woman.

Case Report

A 92-year-old Japanese woman consulted to our hospital because of right nasal obstruction and epistaxis. Physical examination revealed a black tumor measuring of the right nasal cavity. Imaging modalities also showed a tumor in the right nasal cavity (Figure 1). A biopsy was taken, and the biopsy showed malignant epithelioid cells with brown pigment deposition (Figure 2). The brown pigment was positive with Fontana-Masson stain, and thought to be melanin.

An immunohistochemical analysis was performed, using Dako's Envision method, as previously described.[11-13] Immunohistochemically, the tumor cells were positive for S100 protein (Figure 3), HMB45 (Figure 4), p53, Ki-67 (labeling=20%), KIT (Figure 5) and *PDGFRA* (Figure 6). The tumor was negative for cytokeratins (AE1/3 and CAM5.2).

Genetic analyses of the *KIT* gene (exons 9, 11, 13, and 17) and the *PDGFRA* (exons 12 and 18) gene were performed by the PCR direct sequencing method, as previously reported.[13-17] The exons of both genes were selected because

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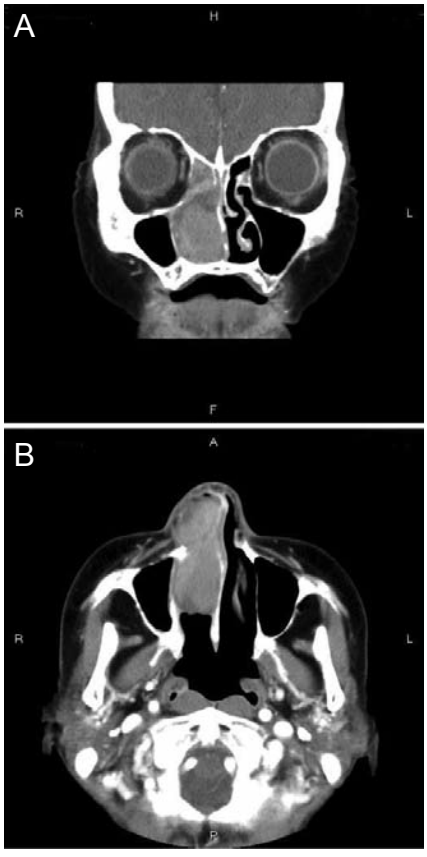

Figure 1. Computed tomography demonstrate obstructing tumor in the right nasal cavity: A) frontal section; B) coronal section.

they are frequent mutation sites.[3] The primers are shown in Table 1. In brief, genomic DNA was extracted from paraffin blocks with proteinase K digestion and phenol/chloroform extraction, and subjected to PCR for 40 cycles (94°C for one minute, 52°C for one minute, 72°C for one minute), using a thermal cycler (GeneAmp PCR system 9700, Applied Biosystems, ABI, CA). The annealing temperature was 53°C. PCR products were extracted, and subjected to a computed automatic DNA sequencer (ABI PRISM 3100 Genetic Analyzer, Applied Biosystems, ABI, CA). These techniques revealed that there were no mutations of the KIT gene (exons 9, 11, 13, and 17) or *PDGFRA* gene (exons 12 and 18) in this tumor.

The pathological diagnosis was primary nasal melanoma. The tumor was resected as far as possible by operation. The tumor was reduced in size by the operation and chemotherapy (Darthmose regimen: dacarbazine, carmustine, cisplatine, and tamoxifen). Five courses of Dartmouth regimen were performed. Extensive operation, lymph node dissection and radiation therapy were not performed because of the patient's old age (92 years). The patient is now alive free from metastasis 9 months after the first manifestation.

Table 1. Primer sequence

Forward	Reverse
KIT exon 9	
5'-TCC TAG AGT AAG CCA GGG CTT-3'	5'-TGG TAG ACA GAG CCT AAA CAT CC-3'
KIT exon11	
5'-GAT CTA TTT TTC CCT TTC TC-3'	5'AGC CCC TGT TTC ATA CTG AC-3'
KIT exon 13	
5'-GCT TGA CAT CAG TTT GCC AG -3'	5'-AAA GGC AGC TTG GAC ACG GCT TTA-3'
KIT exon 17	
5'-CTC CTC CAA CCT AAT AGT GT-3'	5'-GTC AAG CAG AGA ATG GGT AC-3'
PDGFRA exon12	
5'-TTG GAT ATT CAC CAG TTA CCT GTC-3'	5'-CAA GGG AAA AGC TCT TGG-3'
PDGFRA exon 18	
5'-ACC ATG GAT CAG CCA GTC TT-3'	5'-TGA AGG AGG ATG AGC CTG ACC-3'

Discussion

The present study is the second report of *PDGFRA* protein status in melanoma and is the first in melanoma of the nasal cavity. Our previous study[7] showed 100% expression of *PDGFRA* protein in cutaneous melanoma. The present study is the forth report of *PDGFRA* mutations in melanoma; the first was reported by Curtin *et al.*,[5] who found no *PDGFRA* mutations in 26 cutaneous melanomas. The second was reported by Sihto *et al.*,[18] who demonstrated no *PDGFRA* gene mutations in 14 cutaneous melanomas. The third was reported by us; no mutations of *PDGFRA* gene were found in 12 cutaneous melanoma. The present case is the first report of *PDGFRA* gene status in the nasal melanoma. The present case suggests that *PDGFRA* protein is expressed in nasal melanoma, but *PDGFRA* gene mutation is absent in nasal melanoma.

The present case showed no mutations of the *KIT* gene. Studies of *KIT* mutations are scant in number in cutaneous melanoma, and there are none in nasal melanoma. Willmore-Payne *et al.*[19] showed only 2% of melanomas had *KIT* mutations. Sihto *et al.*[18] showed no *KIT* mutation in 14 cutaneous melanomas. In contrast, Curtin *et al.*[1] showed that *KIT* mutations are present in 39% of mucosal melanomas, in 36% of acral melanomas, 28% in melanomas of sun-damaged skin, and in 0% of melanomas of non-sun-damaged skin. Beadling *et al.*[20] recently reported that *KIT* mutations were present in 23% of acral melanomas, 15.6% of mucosal melanomas, 1.7% of cutaneous melanomas, 7.7% of conjunctival melanomas, and 0% of choroidal melanomas. Handolias *et al.*[21] reported that *KIT* mutation was present in 2% of melanomas and that *KIT* mutation is frequent in acral and sun-damaged skin melanomas and mucosal melanomas while it was very rare in non-sun-damaged skin melanoma. In the present case,

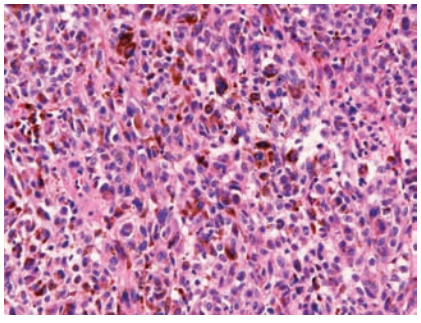

Figure 2. Histology of the conjunctival tumor. Malignant epitheloid cells are seen. Brown pigment was present. These features are suspicious of conjunctival melanoma. Hematoxyne and Eosine x200.

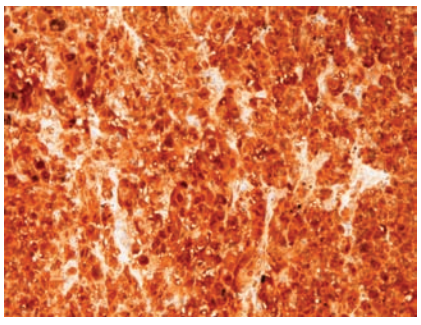

Figure 3. The tumor cells are positive for S100 protein. Immunostaining x200.

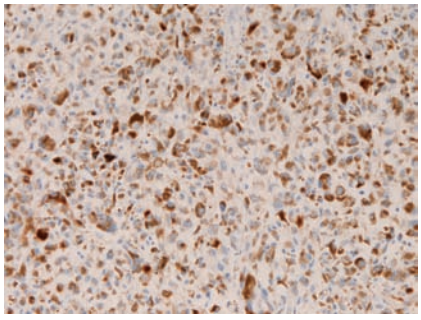

Figure 4. The tumor cells are positive for HMB45. Immunostaining, x200.

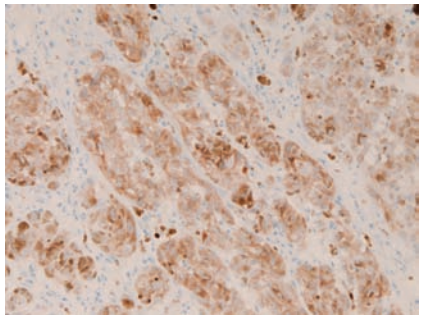

Figure 5. The tumor cells are positive for KIT protein. Immunostaining, x200.

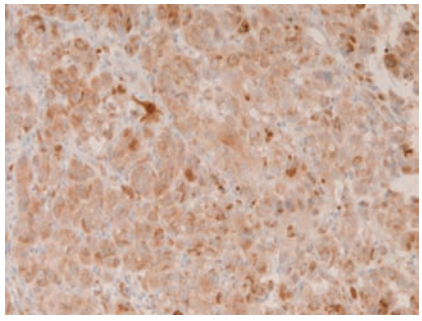

Figure 6. The tumor cells are positive for PDGFRA. Immunostaining, x200.

no mutations were seen in the *KIT* gene. Since *KIT* mutational studies are scant in nasal melanoma, more studies remain to be performed.

The present case showed positive KIT protein expression in nasal melanoma. The percentage of KIT expression in cutaneous melanomas varies among researchers. There have been no reports of KIT expression in nasal melanoma, to the best of our knowledge. The percentage in the literature ranges from 21%[22] to 84%.[23] Sihto *et al*.[18] reported that *KIT* expression in most human solid tumors, including melanomas, were due to KIT gene amplification. More studies of the relationship between KIT gene mutations and KIT protein expression in nasal melanoma remain to be performed.

In conclusion, the author reported a case of melanoma of the nasal cavity expressing KIT and PDGFRA proteins without gene mutations of KIT and *PDGFRA*.

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