Malignant melanoma of the conjunctiva: a case report with examination of *KIT* and *PDGFRA*

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Abstract

Although many clinicopathological studies of malignant melanoma of the conjunctiva have been reported, there have been no studies of the expression and gene mutations of *KIT* and *PDGFRA* in melanoma of the conjunctiva. A 69-year-old Japanese woman consulted our hospital because of black mass (0.7 x 0.7 x 0.6 cm) in the conjunctiva. A biopsy was taken. The biopsy showed malignant epithelioid cells with melanin deposition. Immunohistochemically, the tumor was positive for S100 protein, HMB45, p53, Ki-67 (labeling=30%), KIT and PDGFRA. The tumor was negative for pancytokeratins (AE1/3 and CAM5.2). A genetic analysis using PCR-direct sequencing revealed no mutations of *KIT* gene (exons 9, 11, 13, and 17) and *PDGFRA* gene (exons 12 and 18). The pathological diagnosis was conjunctival melanoma. Despite chemotherapy, the patient developed multiple metastases of melanoma, and died of melanoma 7 years after the biopsy. In conclusion, the author reported a case of melanoma of conjunctive expressing KIT and PDGFRA proteins 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] is available in Mongoloids, including Japanese in which malignant melanoma is much more uncommon than in Caucasians.[9] Ashida *et al.*[6] reported that KIT protein expression was 48% in Japanese cutaneous melanoma and that *KIT* mutations were 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 many clinicopathological studies on melanoma of conjunctiva have been performed,[10,11] there have been no studies of *KIT* and *PDGFRA* in melanoma of the conjunctiva. The author investigated the protein expression and gene mutation status of *KIT* and *PDGFRA* in a case of conjunctival melanoma of a Japanese woman.

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Case Report

A 69-year-old Japanese woman consulted our hospital because of black mass in the conjunctiva. Physical examination revealed a black tumor measuring 0.7×0.7×0.6 cm of the right conjunctiva. A biopsy was taken, and the biopsy showed malignant epithelioid cells with brown pigment deposition (Figure 1). The brown pigment was positive with Fontana-Masson stain, and therefore was thought to be melanin. An immunohistochemical analysis was performed, using Dako's Envision method, as previously described.[12-14] Immunohistochemically, the tumor cells were positive for S100 protein (Figure 2), HMB45 (Figure 3), p53, Ki-67 (labeling=30%), KIT (Figure 4) and PDGFRA (Figure 5). The tumor was negative for pancytokeratins (AE1/3 and CAM5.2).

Genetic analyses of the *KIT* gene (exons 9, 11, 13, and 17) and *PDGFRA* (exons 12 and 18) gene were performed by the PCR direct sequencing method, as previously reported.[15-19] The exons of both genes were selected because 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 min, 52°C for one min, 72°C for one min), 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) and *PDGFRA* gene (exons 12 and 18) in this tumor.

The pathological diagnosis was conjunctival melanoma. Despite chemotherapy, the patient developed multiple metastases of melanoma, and died of melanoma 7 years after the biopsy.

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 case is the second report of PDGFRA protein status in melanoma and is the first in conjunctival melanoma. 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 *PDGFR* mutations in 26 cutaneous melanomas. The second was reported by Sihto *et al.*,[20] 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 melanomas. The current case is the first report of PDGFRA gene status in the conjunctival melanoma.

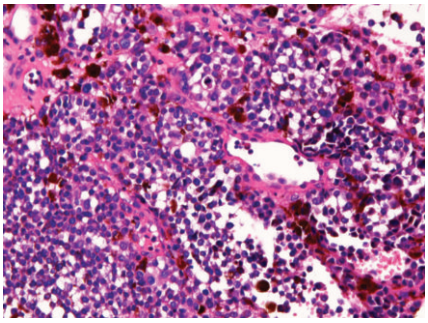

Figure 1. Histology of the conjunctival tumor. Malignant epitheloid cells are seen. Brown pigment was present. These features are suspicious of conjunctival melanoma. Haematoxylin & Eosin, x200.

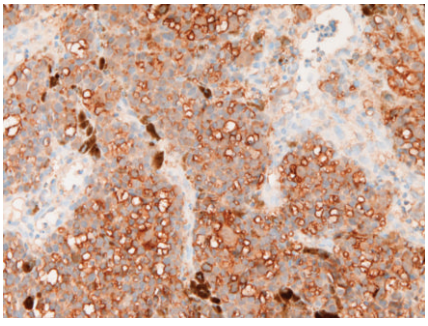

Figure 2. The tumor cells are positive for S100 protein. Immunostaining, x200.

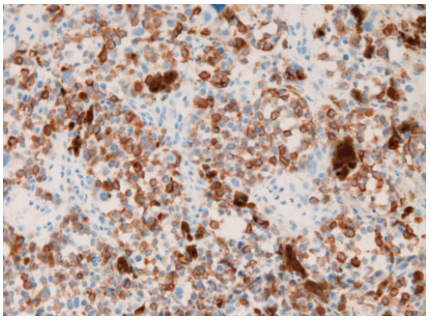

Figure 3. The tumor cells are positive for HMB45. Immunostaining, x200.

The present case showed no mutations of the *KIT* gene. Studies of *KIT* mutations are scant in number in cutaneous melanoma, and are none in conjunctival melanoma. Willmore-Payne *et al.*[21] showed only 2% of melanomas had *KIT* mutations. Sihto *et al.*[20] showed no *KIT* mutations in 14 cutaneous melanomas. In contrast, Curtin *et al.*[1] showed that *KIT* mutations were 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.*[22] recently reported that *KIT* mutations were present in 23% of acral melanomas, 15.6% of mucosal melanomas, 1.7% of cutaneous melanomas, and 0% of choroidal melanomas. Handolias *et al.*[23] reported that *KIT* mutations were present in 2% of melanomas and that *KIT* mutations were 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, no mutations were seen in the *KIT* gene. Since *KIT* mutational studies are scant in conjunctival melanoma, more studies remain to be performed.

The present case showed positive KIT protein expression in conjuctival melanoma. The percentage of KIT expression in cutaneous melanomas varies among researchers. There have been no reports of KIT expression in conjunctival melanoma, to the best of our knowledge. The percentage in the literature ranges from 21 %[24] to 84%.[25] Sihto *et al.*[20] 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 conjunctival melanoma remain to be performed.

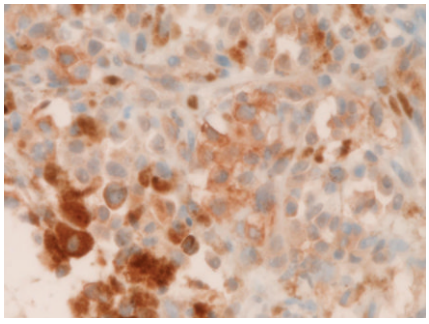

Figure 4. The tumor cells are positive for KIT protein. Immunostaining, x300.

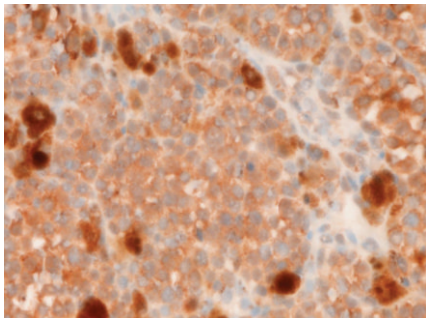

Figure 5. The tumor cells are positive for PDGFRA. Immunostaining, x300.

In conclusion, the author reported a case of melanoma of conjunctiva expressing KIT and PDGFRA proteins without gene mutations of *KIT* and *PDGFRA*. Because this is only a case report, examinations of larger number of patients are needed.

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