Malignant melanoma of the conjunctiva: a case report with examination of KIT and PDGFR A

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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 PDGFR A 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 PDGFR A. 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 PDGFR A 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 conjunctival expressing KIT and PDGFR A proteins without gene mutations of KIT and PDGFR A.

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 x 0.7 x 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 PDGFR A (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 PDGFR A (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.2 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 PDGFR A 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 CTT TC-3’ | 5’-GCC CCC TGT TTC ATA CTG AC-3’ |
| KIT exon 13 5’-GCT TGA CAT CAG TTT GCC AG-3’ | 5’-AAA GGC AGC TGG GAC AGC GCT TTA-3’ |
| KIT exon 17 5’-CTC CTC CCA CCT AAT AGT GT-3’ | 5’-GTC AAG CAG CAG ATG GGT AC-3’ |
| PDGFR A exon 12 5’-TIG GAT ATT CAC CAG TTA CTC GTC-3’ | 5’-CAA GGG AAA GAC TCT TGG G-3’ |
| PDGFR A exon 18 5’-ACC ATG GAT CAG CCA GTC TT-3’ | 5’-TGA AGG AGG ATG ACC 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 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. who found no PDGFRA gene mutations in 26 cutaneous melanomas. The second was reported by Sihto et al. 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 melanomas. The current case is the first report of PDGFRA gene status in conjunctival melanoma.

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. showed only 2% of melanomas had KIT mutations. Sihto et al. showed no KIT mutations in 14 cutaneous melanomas. In contrast, Curtin et al. 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. 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. 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 conjunctival 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% to 84%. Sihto et al. 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.

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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