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doi:10.3748/wjg.15.2679

World J Gastroenterol 2009 June 7; 15(21): 2679-2683
World Journal of Gastroenterology ISSN 1007-9327

CASE REPORT

Amelanotic malignant melanoma of the esophagus: Report of two cases with immunohistochemical and molecular genetic study of *KIT* and *PDGFRA*

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Received: February 6, 2009 Revised: April 13, 2009
Accepted: April 20, 2009
Published online: June 7, 2009

Abstract

The author reports herein two cases of amelanotic malignant melanoma of the esophagus. Case 1 is an 87-year-old woman who was admitted to our hospital because of nausea and vomiting. Endoscopic examination revealed an ulcerated tumor of the distal esophagus, and a biopsy was taken. The biopsy showed malignant polygonal and spindle cells. No melanin pigment was recognized. Immunohistochemically, the tumor cells were positive for melanosome (HMB45), S100 protein, KIT and Platelet derived growth factor receptor-α (PDGFRA). The patient was treated by chemotherapy and radiation, but died of systemic metastasis 12 mo after the presentation. Case 2 is a 56-year-old man presenting with dysphagia. Endoscopic examination revealed a polypoid tumor in the middle esophagus, and a biopsy was obtained. The biopsy showed malignant spindle cells without melanin pigment. Immunohistochemically, the tumor cells were positively labeled for melanosome, S100 protein, KIT and PDGFRA. The patient refused operation, and was treated by palliative chemotherapy and radiation. He died of metastasis 7 mo after the admission. In both cases, molecular genetic analyses of *KIT* gene (exons 9, 11, 13 and 17) and *PDGFRA* gene (exons 12 and 18) were performed by the PCR direct sequencing method, which showed no mutations of *KIT* and *PDGFRA* genes. This is the first report of esophageal malignant melanoma with an examination of the expression of KIT and PDGFRA and the mutational status of *KIT* and *PDGFRA* genes.

Key words: Esophagus; Amelanotic melanoma; KIT; Platelet derived growth factor receptor-α

Peer reviewers: Kyoichi Adachi, MD, Department of Gastroenterology and Hepatology, Shimane University, School of Medicine Shimane, 89-1 Enya-cho, Izumo-shi Shimane 693-8501, Japan; Jia-Yu Xu, Professor, Rui Jin Hospital, Shanghai Jiao Tong University School of Medicine, 197 Rui Jin Er Road, Shanghai 200025, China

Terada T. Amelanotic malignant melanoma of the esophagus: Report of two cases with immunohistochemical and molecular genetic study of *KIT* and *PDGFRA*. *World J Gastroenterol* 2009; 15(21): 2679-2683 Available from: URL: http://www.wjgnet.com/1007-9327/15/2679.asp DOI: http://dx.doi.org/10.3748/wjg.15.2679

INTRODUCTION

Primary malignant melanoma of the esophagus is very rare; only case reports and studies of small series have been reported[1-7]. Primary amelanotic malignant melanoma of the esophagus is extremely rare; only a few case reports have been published in the literature[8-13].

Melanoma is a highly aggressive tumor, and *NRAS* and *BRAF* mutations are mainly involved in the pathogenesis of melanoma[14,15]. *KIT* gene, mapped to 4q12, encodes an oncogenic transmembranous receptor tyrosine kinase, KIT, whose ligand is stem cell factor[6-21]. The *platelet derived growth factor receptor-α* (*PDGFRA*) gene, also mapped to 4q12, additionally encodes an oncogenic transmembranous receptor tyrosine kinase, PDGFRA[6-21]. The *KIT* gene plays an important role in melanocyte migration, development, differentiation and tumorigenesis[22]. A few previous studies have shown that activating mutations of the *KIT* gene may lead to tumorigenesis of cutaneous melanoma[14,23]. Since both *KIT* and *PDGFRA* genes are mapped to 4q12, it is anticipated that *PDGFRA* gene mutations are also involved in the tumorigenesis of melanoma, as in the case of gastrointestinal stromal tumors[16-21]. However, the incidence of *PDGFRA* gene mutations in melanoma has rarely been estimated[24].

The author herein reports two cases of esophageal amelanotic malignant melanoma with immunohistochemical and molecular genetic study of *KIT* and *PDGFRA*.

CASE REPORT

Case 1

An 87-year-old woman was admitted to our hospital because of nausea and vomiting. Endoscopic examination revealed an ulcerated tumor of the distal esophagus, and a biopsy was taken. The biopsy was stained with HE. An immunohistochemical analysis was performed, using Dako's Envision method, as previously described[25-30].

Genetic analyses of the *KIT* gene (exons 9, 11, 13 and 17) and the *PDGFRA* gene (exons 12 and 18) were performed by the PCR direct sequencing method, as previously reported[31-35]. The exons of both genes were selected because they are frequent mutation sites[16-21]. 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℃ for 1 min, 52℃ for 1 min, 72℃ for 1 min), using a thermal cycler (GeneAmp PCR system 9700, Applied Biosystems, ABI, CA). The annealing temperature was 53℃. PCR products were extracted, and subjected to computed automatic DNA sequencing (ABI PRIZM 3100 Genetic Analyzer, Applied Biosystems, ABI, CA).

The biopsy showed malignant spindle and polygonal cells (Figure 1A and B) suspicious for sarcoma. No melanin pigment was recognized by HE and Masson-Fontana stains. The histology was relatively uniform in the biopsy. Immunohistochemically, the malignant cells were positive for melanosome (Figure 1C) (HMB45, Dako), S100 protein (Figure 1D) (polyclonal, Dako), vimentin (Vim 3B4, Dako), p53 protein (DO-7, Dako), neuron-specific enolase (BBS/NC/VI-H14, Dako), PDGFRA (Santa Cruz, CA, USA), and KIT (polyclonal, Dako) (Figure 1E). The KIT expression was focal. In contrast, the malignant cells were negative for cytokeratins (AE1/3 and polyclonal, Dako), CD3 (M7193, Dako), CD10 (M0727, Dako), CD15 (M0733, Dako), CD30 (M0751, Dako), CD45 (M0855, DAKO), CD45RO (UCHL-1, Dako, CD79α (M7050, Dako), CD20 (L26, Dako), desmin (D33, Dako), α-smooth muscle actin (1A4), CD34 (QBEND10, Dako), chromogranin (DAK-A3, Dako), synaptophysin (polyclonal, Dako), CD56 (MOC-1, Dako), and myoglobin (polyclonal, Dako). Ki-67 labeling (MIB1, Dako) was 80%. A pathologic diagnosis of amelanotic melanoma of the esophagus was made. The molecular genetic analysis showed no mutation of the *KIT* gene (exons 9, 11, 13 and 17) or the *PDGFRA* gene (exons 12 and 18). Examination of the skin, eye and intestine showed no tumors. Therefore, the esophageal melanoma was primary. The patient was inoperative because of weakness and old age, and chemotherapy and radiation were performed. The patient showed systemic metastasis, and died of respiratory failure 12 mo after the first presentation. One additional biopsy of the lung metastasis was obtained, and it showed amelanotic melanoma histology and immunohistochemistry results which were almost the same as those of the primary esophageal biopsy.

Table 1 Primer sequence

	Forward	Reverse
KIT exon 9	5′-TCCTAGAGTAAG CCAGGGCTT-3′	5′-TGGTAGACAGAG CCTAAACATCC-3′
KIT exon11	5′-GATCTATTTTTC CCTTTCTC-3′	5′AGCCCCTGTTTCATA CTGAC-3′
KIT exon 13	5′-GCTTGACATCAG TTTGCCAG-3′	5′-AAAGGCAGCTTG GACACGGCTTTA-3′
KIT exon 17	5′-CTCCTCCAACCT AATAGTGT-3′	5′-GTCAAGCAGAGA ATGGGTAC-3′
PDGFRA exon12	5′-TTGGATATTCAC CAGTTACCTGTC-3′	5′-CAAGGGAAAAGC TCTTGG-3′
PDGFRA exon 18	5′-ACCATGGATCAG CCAGTCTT-3′	5′-TGAAGGAGGATG AGCCTGACC-3′

Case 2

A 56-year-old man presented with dysphagia. Endoscopic examination revealed a polypoid tumor in the middle esophagus. A biopsy was taken (Figure 2A and B). Histologically, the biopsy showed proliferation of malignant spindle cells (Figure 2A and B). No melanin pigment was seen with HE and Masson-Fontana stains. The histology of the tumor was relatively uniform in the biopsy. Immunohistochemically, the tumor cells were positive for melanosome (Figure 2C), S100 protein, vimentin, p53 protein, PDGFRA, and KIT (Figure 2D). The KIT expression was diffuse. In contrast, the malignant cells were negative for cytokeratins, CD3, CD30, CD45, CD45RO, CD79α, CD20, desmin, α-smooth muscle actin, CD34, chromogranin, synaptophysin, CD56, and myoglobin. Ki-67 labeling was 95%. A pathological diagnosis of amelanotic malignant melanoma was made. The molecular genetic analysis identified no mutation of either *KIT* gene (exons 9, 11, 13 and 17) or *PDGFRA* gene (exons 12 and 18). No tumors were identified in the skin, eye and mucosal membrane. Therefore, the esophageal melanoma was considered primary. The patient refused operation, and was treated with palliative chemotherapy and radiation. The patient later showed systemic metastasis and died of melanoma 7 mo after the first presentation. No histological specimens were obtained from the metastatic sites.

DISCUSSION

Amelanotic melanoma of the esophagus is extremely rare, and the pathological diagnosis is very difficult because melanin pigment is absent. In fact, it has been frequently misdiagnosed as other sarcomas[1-13]. Immunohistochemistry of melanoma antigens such as melanosome (HMB45), Malan-A, and S100 protein are mandatory for the diagnosis of amelanotic melanoma. Ultrastructural demonstration of melanosome is also diagnostic. In the present study, both cases were positive for immunoreactive melanosome (HMB45) and S100 protein, confirming the diagnosis. In addition, positive KIT strongly suggests that the tumors are melanomas. Amelanotic melanoma can be diagnosed in biopsy specimens.

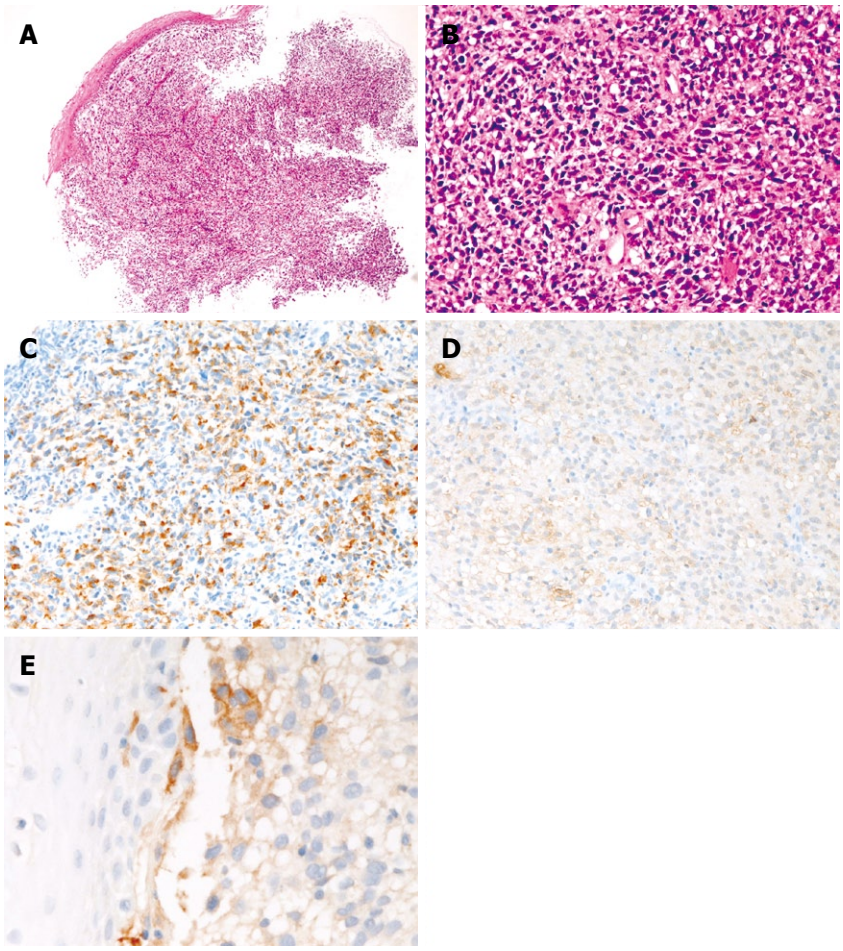

Figure 1 Esophageal amelanotic malignant melanoma in Case 1. A: Low power view of the biopsy (HE, × 20); B: High power view of the biopsy. Malignant polygonal and spindle cells are seen. No melanin pigment is seen (HE, × 200); C: The tumor cells are positive for melanosome (HMB45). (Immunostaining, × 200); D: The tumor cells are positive for S100 protein (Immunostaining, × 200); E: The tumor cells are focally positive for KIT (Immunostaining, × 200).

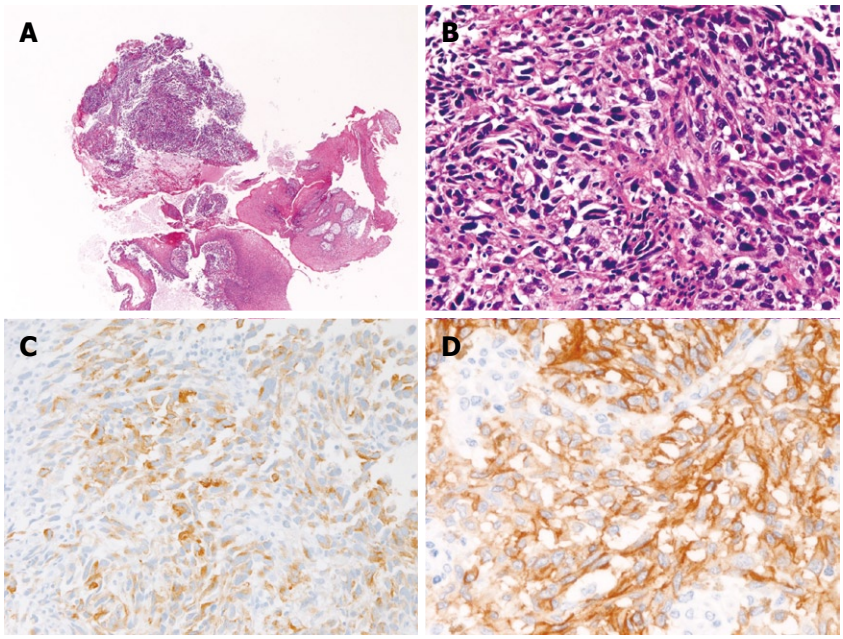

Figure 2 Esophageal amelanotic malignant melanoma in Case 2. A: Low power view of the biopsy (HE, × 20); B: High power view of the biopsy. Malignant spindle cells are seen. No melanin pigment is seen (HE, × 200); C: The tumor cells are positive for melanosome (HMB45) (Immunostaining, × 200); D: The tumor cells are diffusely positive for KIT (Immunostaining, × 200).

Malignant melanoma is a very aggressive tumor irrespective of its location. Malignant melanoma of the esophagus is therefore a very aggressive tumor, and its prognosis is very poor[1-13]. In the present study, the prognosis was indeed very poor in both patients. Operation followed by adjuvant chemotherapy and radiation is the best choice of treatment[1-13]. In the present cases, operation was impossible in one case and was not performed in another case because of the patient's decision not to proceed.

The previously reported cases of esophageal melanoma were only case reports or clinical studies of very small series. There have been no reports of *KIT* and *PDGFRA* expression and mutations in esophageal melanomas. The present study is the first report of esophageal melanoma with an examination of KIT and PDG-

FRA protein expression and gene status of *KIT* and *PDGFRA* in esophageal melanoma. The tumors in the present cases expressed KIT and PDGFRA, but identified no mutations of *KIT* and *PDGFRA*. The positive expressions of KIT and PDGFRA suggest that these transmembranous oncoproteins are present in esophageal melanoma.

In cutaneous melanomas, the percentage of KIT expression varies amongst studies reported by researchers[36]. The percentage of KIT positive cutaneous melanomas in the literature is as follows; 35%[37], 21%[38], 87%[39], 90%[40], 50%[41] and 84%[42]. Sihto *et al*[36] reported that KIT expression in most human solid tumors, including cutaneous melanomas, was due to *KIT* gene amplification. Studies of *KIT* mutations in cutaneous melanoma are scant. Willmore-Payne *et al*[23] showed only 2% of *KIT* mutations in cutaneous melanomas. Sihto *et al*[36] showed no *KIT* mutations in 14 cutaneous melanomas. In contrast, Curtin *et al*[14] showed that *KIT* mutations are present in 39% of mucosal melanomas, in 36% of acral melanomas, in 28% of melanomas on sun-damaged skin, and in 0% of melanomas on non-sun-damaged skin. Beadling *et al*[37] recently reported that *KIT* mutations were present in 23% of acral melanomas, 15.6% of mucosal melanomas, 7.7% of conjunctival melanomas, 1.7% of cutaneous melanoma, and in 0% of choroidal melanomas.

PDGFRA protein expression in melanoma has not been performed, to the best of the author's knowledge. As for *PDGFRA* mutations, Curtin *et al*[24] found no *PDGFRA* mutations in 26 cutaneous melanomas. Sihto *et al*[36] demonstrated no *PDGFRA* gene mutations in 14 cutaneous melanomas.

In summary, the author reported two extremely rare cases of amelanotic malignant melanoma of the esophagus with immunohistochemical and genetic analysis of *KIT* and *PDGFRA*.

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