



Primary malignant melanoma of esophagus: clinicopathologic characterization of 20 cases including molecular genetic profiling of 15 tumors

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Abstract

Primary malignant melanoma of esophagus is very rare, and its clinicopathologic and genetic features have not been extensively investigated. In this study, 20 tumors from 14 male and 6 female patients (40–79 years old) were evaluated. Dysphagia, chest pain, and weight loss were frequent symptoms. Thirteen melanomas, including two with multiple lesions, involved the distal third of esophagus. The median tumor diameter was 6 cm. Epithelioid morphology, moderate atypia, and pigmentation were typical findings. None of the patients had melanoma elsewhere, and all tumors exhibited a junctional peri-epithelial component consistent with a primary lesion. The median mitotic activity was 11 per 10 high-power fields (range, 0–31). Nine patients died of tumor within 4–22 months, however, two showed long-term (96 and 104 months) survival. In 15 cases, tissue for further immunohistochemical and molecular studies were available. *BRAF*, *KIT*, and *NRAS* mutation status was assessed by Sanger sequencing in all 15 tumors. The next-generation sequencing of 50 or 409 genes was performed in five and three cases, respectively. IGF1R expression indicating activation of the IGF axis was seen in 82% (9/11) of tumors. However, no *BRAF* mutations were identified. In 33% (5/15) of tumors, *NRAS* mutations were detected. *KIT* expression was seen in 50% (7/14) of melanomas including single *KIT* mutant. Two of three tumors evaluated with 409 genes panel revealed multiple driver mutations indicating sub-clonal expansion, whereas a single mutation (*TSC1* p.H371Q) was the sole change in the third case. *SF3B1* p.K666T and p.R625C mutations were detected in two cases. However, no co-occurrence of *SF3B1* and *GNAQ* or *GNAI1* mutations, seen in uveal melanoma, was detected. *FBXW7* p.R465C and p.R479G mutations, linked to cancer progression, were found in two of eight tumors. In summary, esophageal melanoma mutation profile indicates complexity of molecular mechanisms underlying its pathogenesis.

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Introduction

Primary malignant melanoma of esophagus (from here on called “esophageal melanoma”) is an extremely rare neoplasm with the incidence estimated to be 0.03 per million in the USA [1]. Because of its rarity, only a few small series of esophageal melanoma are available [2–6] and most clinicopathologic data are derived from > 300 single case studies published since the first histologic description [7–9].

Esophageal melanoma is believed to develop from melanocytes anchored in the esophageal mucosa. Aberrant migration of melanoblasts to the esophagus can occur during their early migration from the neural crest to the epidermis and other sites [10–12]. Primary esophageal melanoma should not be confused with metastatic melanoma, which could present in any portion of the gastrointestinal tract. Although involvement of esophagus is

uncommon, the differential diagnosis between metastatic and primary melanoma can be challenging [5, 13, 14]. Metastatic melanoma cells can infiltrate mucosa mimicking primary junctional changes [15]. Thus, a primary mucosal melanoma should be defined by identification of melanocytes at the epithelial–stromal junction and/or an adjacent melanoma in situ, and lack of primary cutaneous melanoma [16].

The mutation profile of esophageal melanoma remains incompletely characterized. Sanger sequencing data available of 30 cases are limited to *BRAF*, *NRAS*, and *KIT* mutation status [17–20]. More recent studies have employed next-generation sequencing for multiple targets, or whole-genome sequencing, on a few primary and metastatic tumors [21–24]. However, those studies did not clearly state the diagnostic criteria for primary esophageal melanoma so that metastatic melanomas might have been included.

The aim of this study was to evaluate the clinicopathologic and molecular genetic profile of 20 strictly defined primary esophageal melanomas containing junctional melanocytes.

Materials and methods

This study evaluated a series of 20 primary malignant melanomas of esophagus. Sixteen tumors were from the Armed Forces Institute of Pathology, Washington, D.C. Additional four tumors were contributed by co-authors. Demographic, clinical, and follow-up data were obtained according to the Institutional Review Board approvals.

Immunohistochemical studies

Expression of several antigens including melanocytic differentiation markers (human melanoma black [HMB]–45, KBA.62-melanoma associated antigen, Melan-A protein, microphthalmia-associated transcription factor [MITF], PNL-2-melanoma associated antigen, S100 protein, tyrosinase [TYR]) and CD34, CD117 (KIT), cytokeratin 8 (CK8), Cytokeratin cocktail (AE1/AE3), DOG1 (discovered on GIST1; also known as anoctamin 1, or ANO1), insulin-like growth factor 1 receptor (IGF1R), and Vimentin was evaluated immunohistochemically. Leica Bond-Max automated immunostainer (Leica, Bannockburn, IL) was used in this study. Detailed description of antibodies and immunohistochemical protocols is provided in supplemental data.

Molecular genetic studies

In 15 cases, formalin-fixed paraffin-embedded tissue blocks or unstained tumor sections were available. DNA was

extracted from 5 to 10 5 μ sections using Maxwell[®] RSC DNA FFPE kit and Maxwell[®] RSC instrument (Promega, Madison, WI) following manufacturer's protocol provided at www.promega.com. All 15 tumors were screened for *BRAF*, *KIT*, and *NRAS* mutations by PCR amplification and Sanger sequencing following previously published protocols [25, 26]. Subsequently, eight tumors with better-preserved DNA (cases 1, 6, 7, 8, 9, 12, 14, and 20) were evaluated by targeted next-generation sequencing. Ion Torrent[™] (Life Technologies/Thermo Fisher Scientific, Waltham, MA) next-generation sequencing platform was used following manufacturer's recommendations. Depending on the DNA quality either Ion AmpliSeq[™] Cancer Hotspot Panel v2 Kit (50 gene targets) or Ion AmpliSeq[™] Comprehensive Cancer Panel (409 gene targets) was employed to evaluate five and three tumors, respectively. Fifty genes targeted by the Cancer Hotspot Panel were included in the Comprehensive Cancer Panel. A list of all genes analyzed in this study is provided in supplemental data.

The data were processed by Torrent Server Suite 4.2 and sequences aligned to human genome reference sequence HG-19 (The Genome Reference Consortium). Variant calling was performed using Variant Caller v4.2, which is compatible with the Integrative Genomics Viewer (Broad Institute, Cambridge, MA), a high-performance visualization tool for interactive exploration of large, integrated data sets. Mutation nomenclature is based on Human Genome Mutation Society (www.hgvs.org) recommendations. The FATHMM (Functional Analysis Through Hidden Markov Models) scores predicting functional consequences of coding variants were obtained from the COSMIC (Catalog of Somatic Mutations in Cancer) at <https://cancer.sanger.ac.uk> or assessed using VarSome (The Human Genomic Variant Search Engine) at <https://varsome.com>.

Results

Demographic and clinicopathologic data

There were 14 males and 6 females (ratio 2.3:1). The median age at the diagnosis was 60 years for men and 63.5 years for women. Caucasian ethnicity was known in 16 cases. Demographic and clinicopathologic data are summarized in Table 1. Clinicopathologic characteristics of cases 5 and 8 were previously published [27, 28]. The latter was metachronous melanoma diagnosed 67 months after successful treatment of primary gastric melanoma located in the cardia [28]. Symptoms preceding the diagnosis most commonly included progressive dysphagia (87% of the cases), abdominal or chest pain (40%), and substantial weight loss (20%). Distal third of the esophagus was the

Table 1 Demographic and clinicopathologic data of 20 primary esophageal melanomas evaluated in this study

Case	Sex	Age	Symptoms			Tumor location	Tumor size (cm)	Local metastases at the surgery	Follow-up (in months)
			Dysphagia	Pain	Weight loss				
1	M	40	Unknown	Unknown	Unknown	Distal	7 × 3 × 3	No	Alive (96)
2	M	45	Yes	No	No	Distal	5.5	Yes	DOD (19)
3	M	47	Yes	No	No	Mid	Unknown	Unknown	DUNK (18)
4	M	51	Yes	Yes	No	Distal	6 × 3 × 5	Yes	DOD (10)
5*	M	57	Yes	Yes	Yes	Mid	7	No	DOPC
6	M	58	Unknown	Unknown	Unknown	Unknown	3 × 2.5	Yes	DOD (20)
7	M	59	Yes	No	No	Mid to distal	12.5 × 4	Yes	DOD (11)
8*	M	61	Yes	No	No	Mid to distal	0.7	No	DOD (16)
9	M	62	No	yes	No	Distal at E_G junction	5 × 4	Unknown	Unknown
10	M	65	No	Yes	No	Unknown	6 × 6 × 3	No	DOD (4)
11	M	68	Yes	Yes	Yes	Distal	6 × 3.5	Yes	DOD (22)
12	M	72	Unknown	Unknown	Unknown	Distal at E_G junction	2.5 × 2.3 × 1 + two more lesions	No	DUNK (7)
13	M	73	Yes	No	No	Distal at E_G junction	8	Yes	Unknown
14	M	79	Yes	Yes	Yes	Distal	7.5 × 4.5	Yes	DOPC
15	F	53	Yes	No	No	Distal	8 × 1.5	Yes	DOD (16)
16	F	55	Yes	No	No	Distal	4 × 1	Yes	Unknown
17	F	58	Yes	No	No	Distal at E_G junction	12 × 12 × 7.5	Yes	DOD (4)
18	F	69	Unknown	Unknown	Unknown	Distal	Two lesions: 3.5 × 2.5 × 1.5; 3 × 2 × 1	No	DUNK (104)
19	F	71	Unknown	Unknown	Unknown	Unknown	2.1 × 1.3 × 0.7	No	DUNK (8)
20	F	78	Yes	No	No	Distal at E_G junction	7 × 4.5 × 4.5	No	Unknown

DOD died of disease, *DOPC* died of postoperative complications, *DUNK* died of unknown causes, *E_G* esophagogastric, *LTF* lost to follow-up,

* some data previously reported [27, 28]

most common location (13 cases), with five of these tumors seated at the esophagogastric junction. The tumor extended to or was limited to the mid-esophagus in four cases. The location was not specified in the remaining three cases. Two patients had multiple lesions. Most esophageal melanomas formed polypoid and lobulated endophytic masses. Tumor size, available in 19 cases, varied from 0.7 to 12 cm (median 6 cm). Ulceration was seen in 17 of 19 cases with suitable data. Metastases in local lymph nodes were detected at the time of surgery in 50% (9/18) of patients. Yet, in all cases, there was no evidence of co-existing or previous cutaneous melanoma.

Histological features

A junctional peri-epithelial tumor component was present in all cases and at least focal melanin pigmentation in 85% (17/20) of cases. Pagetoid involvement of the overlying squamous epithelium was seen in 32% (6/19) of melanomas. Majority of tumors were composed of epithelioid cells.

One tumor showed predominantly spindle cell morphology, and focal spindle or round cell component was seen in four cases and nuclear pleomorphism in five cases. Mitotic activity per 10 high-power fields (HPFs; 2 mm²) varied from 0 to 31 (median 12). Tumor necrosis was seen in 21% (4/19) and ulceration in 90% (17/19) of cases, respectively. Histopathologic data are summarized in Table 2. Representative histological images are shown in Figs. 1a, 2a. Additional figure illustrating junctional changes (atypical melanocytes disposed as single cells and as irregular nests along the basal layer of the esophageal epithelium) in case 19 is available in supplemental data.

Immunohistochemical features

All analyzed tumors were positive for at least for one marker of melanocytic differentiation (Fig. 2b). IGF1R expression was seen in 83% (9/11) evaluated tumors. Although IGF1R expression pattern was diffuse in all cases (Fig. 1b), intensity of immunohistochemical reactions

Table 2 Histopathologic features of 20 primary esophageal melanomas

Case	Cell type	Pigment	Atypia	Mitoses/10 HPF	Tumor necrosis	Junctional activity	Pagetoid spread	Ulceration
1	Epithelioid to spindle	No	Moderate	11	Yes	Yes	No	Yes
2	Epithelioid	No	Moderate	10	No	Yes	No	Yes
3	Epithelioid	Yes	Moderate	0	No	Yes	No	Yes
4	Epithelioid	Yes	Moderate (focally severe)	15	No	Yes	No	Yes
5*	Epithelioid	Yes	Moderate	6	No	Yes	No	Yes
6	Epithelioid	Yes	Moderate	6	No	Yes	Yes (f)	Yes
7	Epithelioid	Yes	Moderate (focally severe)	14	Yes	Yes	No	Yes
8*	Epithelioid	Yes	Moderate	4	No	Yes	No	No
9	Epithelioid	Yes	Moderate	4	No	Yes	No	Yes
10	Epithelioid	Yes	Moderate	23	No	Yes	Yes	Yes
11 [§]	Epithelioid	Yes	Moderate	n/a	n/a	Yes	n/a	n/a
12	Epithelioid to round cell	Yes	Moderate	31	No	Yes	Yes	Yes
13	Epithelioid to spindle	Yes	Moderate	19	Yes	Yes	No	Yes
14	Epithelioid	Yes	Moderate (focally severe)	9	No	Yes	No	Yes
15	Epithelioid	Yes	Moderate	2	No	Yes	No	Yes
16	Epithelioid	Yes	Moderate	11	Yes	Yes	Yes	Yes
17	Spindle to epithelioid	Yes	Severe	14	No	Yes	No	Yes
18	Epithelioid	No	Moderate (focally severe)	7	No	Yes	Yes (f)	Yes
19	Epithelioid	Yes	Moderate	12	No	Yes	Yes (f)	No
20	Epithelioid	Yes	Moderate	29	No	Yes	No	Yes

HPF high-power fields, *f* focal, *n/a* = not available, [§] diagnostic biopsy, * some data previously reported [27, 28]

varied from weak ($n = 2$) to moderate ($n = 4$) and strong ($n = 3$). Fifty percent (7/14) of esophageal melanomas showed variable KIT expression (Figs. 1c, d) with diffuse, strong immunoreactivity seen in two cases. In general, KIT expression was more prominent in junctional areas. No CD34 or DOG1 expression was detected. One tumor, case 20, revealed cytokeratin immunoreactivity (focal with CK8 antibody and more prominent with AE1/AE3 cytokeratin cocktail antibody). Vimentin was expressed in 89% (8/9) of melanomas. Representative images are shown in Figs. 2c, d. Immunohistochemical results are detailed in Table 3.

Molecular genetic features

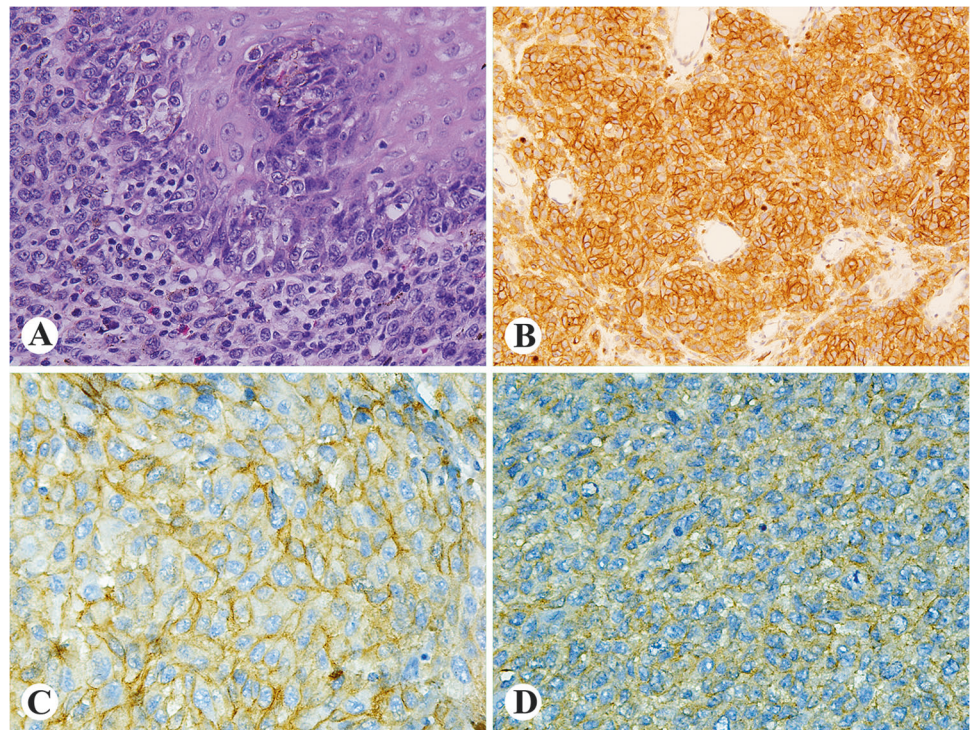
Thirty-three percent (5/15) of tumors harbored *NRAS* mutations. A Q to K ($n = 3$) and Q to H ($n = 1$) substitutions at *NRAS* codon 61 were the most common change. In one case, p.A146T mutations were identified. One tumor contained a *KRAS* codon 13 mutation at relatively (20%) low frequency. Also, in one case, activating *KIT* mutation (p.L576P) was identified.

Two of three cases studied using Ion AmpliSeqTM Comprehensive Cancer Panel next-generation sequencing

contained *SF3B1* (splicing factor 3B subunit 1) mutations (p.R625C and p.K666T). A p.R625C co-occurred with nonsense mutation truncating mammalian target of rapamycin (mTOR) at p.Q1684, whereas p.K666T was detected in melanoma carrying *NRAS* p.Q61K and *KIT* p.L576P driver mutations in addition to cyclin-dependent kinase inhibitor 2B (*CDKN2B*) p.110N and ataxia telangiectasia and Rad3 related (*ATR*) p.V372G substitutions. In one case, tuberous sclerosis 1 (*TSC1*) p.H371Q was the only mutation identified. Two F-box and WD repeat domain containing 7 (*FBXW7*) mutants, p.R465C and p.R479G were found among eight analyzed cases. Tumor characterized by *FBXW7* p.R465C also harbored *KRAS* p.G13C mutations at a low frequency. FATHMM scores describing pathogenicity of the missense variants identified in this study ranged from 0.80 to 0.99 supporting pathogenic potential (complete list provided in supplemental data).

In case 20, *NRAS* p.Q61K and *KIT* WT genotype, and *NRAS* WT and *KIT* p.L576P genotype were identified by Sanger sequencing and next-generation sequencing, respectively. This variation could be related to two different DNA samples evaluated by Sanger and next-generation sequencing.

Fig. 1 Examples of histologic and immunohistochemical findings in esophageal melanoma. Junctional changes (a) and prominent IGF1R expression (b) in case 6. Strong KIT expression in *KIT*-wild type case 7 (c) and weak KIT expression in *KIT* mutant case 20 (d)



No mutations were identified in the genes often indicated in melanoma [29, 30] such as *BRAF* (15 cases analyzed), *CDKN2A*, *GAN11* and *GNAQ*, *PIK3CA* and *TP53* (eight cases analyzed) *BAP1* and *NF1* (three cases analyzed). Sanger and next-generation sequencing results are detailed in Table 4 and supplemental data.

Follow-up data

Metastatic disease at the surgery was diagnosed in 9 of 18 (50%) cases. Follow-up data were available on 16 patients. Two patients including one with local metastases died of postoperative complications. Nine patients died of disease within 4–22 months (mean survival 15 months), while 4 patients died of unknown causes within 8–104 months (mean survival 43 months). One patient was alive without disease 96 months after surgery; two patients, who survived 96 and 104 months, respectively, had no nodal metastases at surgery.

Discussion

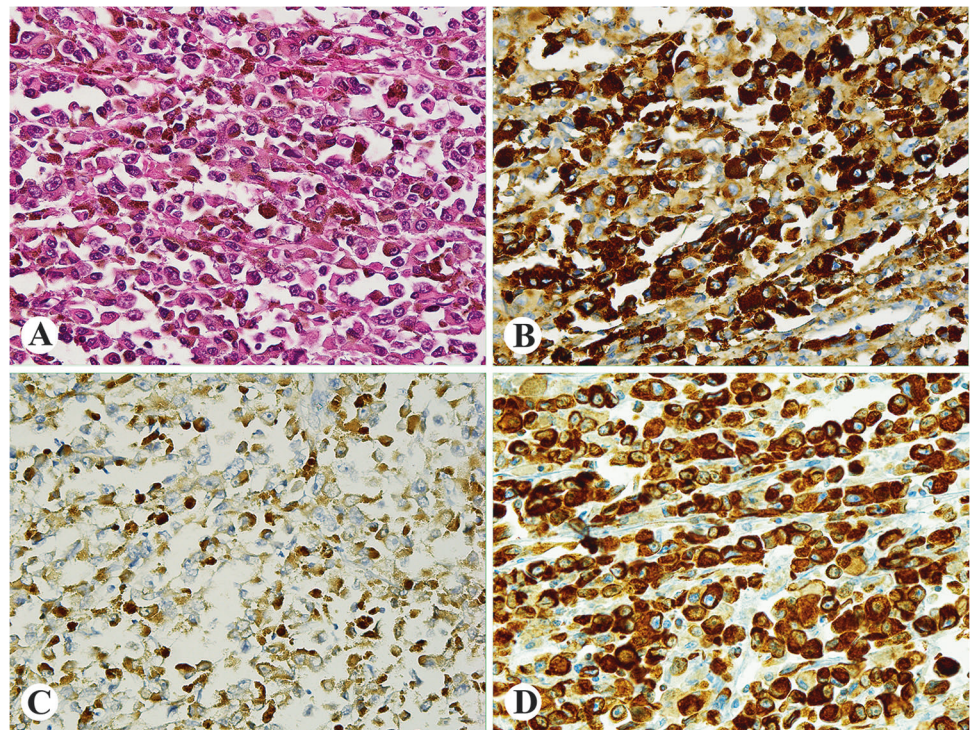
This study analyzed 20 well-documented primary esophageal melanomas. Seventy percent of patients were male. Similar age distribution and predominance of male gender were reported in two recently published largest cohorts of 13 and 17 primary esophageal melanoma patients of Asian ethnicity [31, 32]. Male predominance among primary

esophageal melanoma patients is reportedly not associated with alcohol and tobacco consumption, as seen in esophageal squamous cell carcinoma patients [32]. In this series, most of melanomas arose in the distal third of the esophagus, a location frequently indicated by previous studies [9]. Progressive dysphagia accompanied by upper abdominal pain and weight loss mirror reported main clinical symptoms [33].

Tumor ulceration was seen in almost all (17/19) analyzed esophageal melanomas. Presence of ulceration is a prognostic factor indicating shorter overall-survival for both Stage I and Stage II cutaneous melanoma patients [34]. In this study, one of two cases with long overall survival lacked ulceration. Also, mitotic rate has been considered to be a prognostic factor for cutaneous melanoma [35]. In this study, mitotic rates were slightly higher in esophageal melanomas with overall survival shorter than 12 months.

In general, the prognosis for esophageal melanoma is poor [8, 33]. A great majority of patients included in this series died of disease within several months. However, two long survivals of 104 and 96 months were documented. In both cases, no local lymph node metastases were diagnosed at the curative resection. Patients with esophageal melanoma at T1a stage revealed excellent prognosis compared with more advanced tumors with local lymph nodes metastases [36]. However, long-term survivals (up to 12 years) have been reported in few cases with submucosal invasion and local lymph node metastases treated by subtotal esophagectomy and adjuvant chemotherapy [37, 38].

Fig. 2 Histologic and immunohistochemical findings in case 10. Epithelioid melanoma with moderate atypia and prominent pigmentation (a), strong HMB45 expression (b), showing cytokeratin (c) and vimentin immunoreactivity (d)



In this series of esophageal melanomas, one tumor expressed keratins. This phenomenon has been previously described [39]. The tumor invasiveness and metastatic potential may correlate with keratin and vimentin co-expression [40]. Reported in this study, tumor co-expressing keratin and vimentin showed rapid progression and only 4-month overall survival.

Alterations of proteins forming MAPK (mitogen-activated protein kinase) pathway that communicates signals from cell surface to the nucleus, have been reported in different type of cancers including malignant melanoma [41]. *NRAS* (neuroblastoma RAS viral [v-ras] oncogene homolog) is a member of human RAS proto-oncogene family that encodes cell membrane-associated proteins involved in transduction of extracellular growth and differentiation signals [42]. Typically, oncogenic *NRAS* mutations cluster in exon 1 (G12/13) and exon 2 (Q61) and represent the second most common driver after *BRAF* mutations in melanoma [43]. About 5–20% of mucosal melanoma, depending on tumor location, harbor *NRAS* mutations [44]. In this study, five *NRAS* mutants were identified among 15 esophageal melanomas. Four substitutions were found in codon 61, a “hot-spot” for *NRAS* mutations in melanoma [45], whereas one tumor harbored *NRAS* p.A146T substitution. This mutation has not been reported in melanoma by COSMIC. However, it was identified in melanoma cell line A375 clones with acquired resistance to the dabrafenib GSK2118436, a *BRAF* inhibitor [46]. *NRAS* p.A146T mutation was detected in blastic

plasmacytoid dendritic cell neoplasm, and in B- and T-cell acute lymphoblastic leukemia [47–49].

KIT, a transmembrane receptor tyrosine kinase, plays a crucial role in growth regulation, differentiation, migration, and proliferation of melanocytes. Somatic *KIT* mutations cause oncogenic signaling affecting both the mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3-kinase (PI3K) pathway [50]. *KIT*-mutants were mainly identified among mucosal, acral, and chronically sun-damaged skin tumors [51, 52]. In melanoma, 70% of *KIT* mutations were found in juxtamembrane domain (exon 11) with p.L576P substitution being a most common [53]. This mutation, initially reported in gastrointestinal stromal tumors, leads to pathologic activation of *KIT* tyrosine kinase activity [54]. In this study, p.L576P mutation was found in 7% (1/15) of esophageal melanomas. Previously, a few ($n = 4$) *KIT* mutations including p.L576P, p.H580_G592dup in the juxtamembrane domain, and p.F504L and p.A502_Y503insFA in extracellular domain, were reported in a combined cohort of 17 esophageal melanomas [18, 20, 21]. However, variable *KIT* expression was detected immunohistochemically in a higher number of cases. In this study, 50% of analyzed tumors revealed *KIT* positivity. As reported, *KIT* was stronger expressed in the in situ and junctional component than in the invasive part of the lesion [55]. Although response to tyrosine kinase inhibitors imatinib mesylate and sorafenib in *KIT*-mutated rectal melanoma have been reported in isolated cases [56, 57], larger studies have failed to confirm convincing therapeutic efficacy [58].

Table 3 Immunohistochemical features of 20 primary esophageal melanomas

Case	Melanocytic differentiation markers							Other markers						
	HMB45	KBA.62	Melan-A	MITF	PNL-2	S100	TYR	CD34	CD117 (KIT)	DOG1	IGFR1	Keratin (CK8)	Keratins (pan-CK)	VIM
1	(+)	(+)	(+)	ND	(+)	(+)	(+)	(-)	(+)	ND	(+)	ND	(-)	ND
2	(+)	ND	ND	ND	ND	(+)	ND	ND	ND	ND	ND	ND	ND	ND
3	(+)	ND	ND	ND	ND	(+)	ND	ND	(+)	ND	ND	ND	ND	ND
4	ND	ND	ND	ND	ND	(+)	ND	ND	ND	ND	ND	ND	ND	ND
5	(+)	(-)	(-)	(-)	(+)	(-)	(+)	(-)	(-)	(-)	(+)	(-)	(-)	(+)
6	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(-)	(-)	(-)	(+)	(-)	(-)	(+)
7	(+)	(-)	(+)	(-)	(+)	(+)	(+)	(-)	(+)	(-)	(-)	(-)	(-)	(+)
8	(+)	(-)	(+)	(+)	(+)	(+)	(+)	(-)	(-)	(-)	(+)	(-)	(-)	(+)
9	(-)	(+)	(+)	(+)	(-)	(+)	(-)	(-)	(-)	(-)	(+)	(-)	(-)	(+)
10	(+)	(+)	(+)	(-)	(+)	(+)	(+)	(-)	(-)	(-)	(-)	(+)	(+)	(+)
11	(+)	ND	ND	ND	ND	(+)	ND	ND	(+)	ND	ND	ND	ND	ND
12	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(-)	(+)	(-)	(+)	(-)	(-)	(+)
13	ND	ND	ND	ND	ND	(+)	ND	ND	ND	ND	ND	ND	ND	ND
14	(+)	(-)	(-)	(-)	(+)	(+)	(+)	(-)	(-)	(-)	(+)	(-)	(-)	(-)
15	(+)	ND	ND	ND	ND	(+)	ND	ND	ND	ND	ND	ND	ND	ND
16	(+)	ND	ND	ND	ND	ND	ND	ND	(+)	ND	ND	ND	ND	ND
17	(+)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
18	ND	ND	ND	ND	ND	(+)	ND	ND	ND	ND	ND	ND	ND	ND
19	(+)	(+)	(+)	ND	(+)	(+)	ND	ND	(-)	ND	(+)	(-)	(-)	ND
20	(+)	(-)	(+)	(-)	(+)	(+)	(+)	(-)	(+)	(-)	(+)	(-)	(-)	(+)

ND not done

Two of three esophageal melanomas analyzed with Ion AmpliSeq™ Comprehensive Cancer Panel revealed mutations in the gene encoding splicing factor 3B subunit 1 (SF3B1), a component of the spliceosome. Identical SF3B1 p.R625C and p.K666T mutations were previously reported in uveal melanomas, colorectal and other mucosal melanomas, and have been associated with diverse alternative splicing events [59, 60]. Approximately 20% of each uveal-harbored *SF3B1* somatic mutations. In uveal melanoma, the presence of SF3B1 mutations is associated with mutational activation of GNAQ or GNA11 oncogenes [59]. In esophageal melanoma, no co-occurrence of *SF3B1* and *GNAQ* or *GNA11* mutations was identified. However, one *SF3B1*-mutant harbored KIT p.L576P and NRAS p.Q61K mutations. A recent study documented *SF3B1* mutations in anorectal melanomas harboring *RAS* mutations [22]. Second of *SF3B1*-mutant esophageal melanomas harbored mTOR FAT-domain p.Gln1684* mutation. Previously, missense mTOR mutations were identified in mucosal melanoma and linked to a worse prognosis [61]. Although biological significance of *mTOR* nonsense mutation is unknown, mTOR inactivation may lead to deregulation of mTOR complex 1 and its tumor suppression function [62].

Two esophageal melanomas harbored mutations affecting the *FBXW7* gene. FBXW7 (F-Box and WD repeat

domain containing 7) encodes a member of the F-box protein family. The F-box proteins constitute one of the four subunits of ubiquitin protein ligase complex called SCFs (SKP1-cullin-F-box), which functions in phosphorylation-dependent ubiquitination. A recent melanoma study showed no association between the presence of *FBXW7* and *BRAF* or *RAS* mutations and designated *FBXW7* as a tumor-suppressor gene, a novel driver for a subset of melanomas [63]. In line with this observation, one of the *FBXW7*-mutant esophageal melanomas reported in this study was *BRAF* and *RAS* wild type. However, another tumor harbored a p.G13C *KRAS*-mutant subclone. In general, *KRAS*-mutants are very rare (< 1%) in melanoma and have not been reported in esophageal melanoma. However, concomitant of *FBXW7* and *KRAS* mutations have been found in advanced colorectal carcinomas [64]. Two *FBXW7* mutations identified in esophageal melanoma (p.R465C and p.R479G), which are considered to inactivate *FBXW7* were previously reported in ovarian and head and neck squamous cell carcinomas [64].

In one esophageal melanoma, a p.H371Q mutation in tuberlin-binding domain of *TSC1* was identified as a sole alteration. Recent study reported *TSC1* mutations in a spectrum of mucosal melanomas. In a few *TSC1* mutants, including one esophageal melanoma, alteration of *TSC1*

Table 4 Results of esophageal melanoma genotyping including Sanger sequencing of *BRAF*, *KIT*, and *NRAS* (15 cases) and all mutations identified by the Ion Torrent™ next-generation sequencing with Ion AmpliSeq™ Cancer Hotspot Panel v2 Kit (five cases) and Ion AmpliSeq™ Comprehensive Cancer Panel (three cases)

Case	Methods	Gene									
		<i>ATR</i>	<i>BRAF</i>	<i>CDKN2B</i>	<i>FBXW7</i>	<i>KIT</i>	<i>KRAS</i>	<i>NRAS</i>	<i>MTOR</i>	<i>SF3B1</i>	<i>TSC1</i>
1	Ion AmpliSeq™ CCP Sanger sequencing	WT	WT	WT	WT	WT	WT	WT	WT	WT	p.H371Q
3	Sanger sequencing		WT			WT		WT			
5	Sanger sequencing		WT			WT		WT			
6	Ion AmpliSeq™ CHP Sanger sequencing	NIP	WT		WT	WT	WT	p.Q61K	NIP	NIP	NIP
7	Ion AmpliSeq™ CHP Sanger sequencing	NIP	WT	NIP	p.R479G	WT	WT	WT	NIP	NIP	NIP
8	Ion AmpliSeq™ CHP Sanger sequencing	NIP	WT	NIP	WT	WT	WT	p.A146T	NIP	NIP	NIP
9	Ion AmpliSeq™ CHP Sanger sequencing	NIP	WT	NIP	WT	WT	WT	p.Q61K	NIP	NIP	NIP
10	Sanger sequencing		WT			WT		WT			
11	Sanger sequencing		WT			WT		WT			
12	Ion AmpliSeq™ CCP Sanger sequencing	WT	WT	WT	WT	WT	WT	WT	p.Q1684*	p.R625C	WT
14	Ion AmpliSeq™ CHP Sanger sequencing	NIP	WT	NIP	p.R465C	WT	p.G13C	WT	NIP	NIP	NIP
16	Sanger sequencing		WT			WT		p.Q61H			
17	Sanger sequencing		WT			WT		WT			
19	Sanger sequencing		WT			WT		WT			
20	Ion AmpliSeq™ CCP Sanger sequencing	p.V372G	WT	p.D110N	WT	^x p.L576P	WT	^{xx} p.Q61K	WT	p.K666T	WT

Complete next-generation sequencing data are provided in the Supplemental Table 2 and Supplemental Table 3

CCP Comprehensive Cancer Panel (409 gene targets), CHP Cancer Hotspot Panel v2 Kit (50 gene targets), NIP not included in this panel, ^x next-generation sequencing result, ^{xx} Sanger sequencing result

was the only change and did not co-occur with *NRAS*, *KIT*, or *BRAF* mutations [65]. In contrast with those cases, tumors with multiple driver mutations were seen in this series and previously reported indicating high frequency of somatic mutation in melanoma [66]. Dynamic clonal changes might be responsible for the differences between Sanger and NGS-sequencing results in case 20, especially if different DNA samples are being evaluated.

In this study, no *BRAF* mutations were identified in esophageal melanomas. Previous investigations have reported a small number of *BRAF*-mutant esophageal melanomas [18, 20–24, 66, 67]. However, clinical and histological findings specific for primary esophageal melanoma were not clearly documented, so that the possibility of inclusion of metastatic cutaneous melanoma in those series cannot be excluded. In this study, both atypical junctional changes in the squamous epithelium and/or an adjacent melanoma in situ with no evidence for co-existing or previous cutaneous melanoma was documented in all cases.

In summary, activation of RAS-RAF-MEK pathway through the *NRAS* mutations seems to be essential for

development of a subset of esophageal melanoma, whereas *BRAF* mutations are rare if they occur. Also, mutations of *FBX7*, *KIT*, *SF3B1*, and *TSC1* being previously found in other mucosal melanomas may play significant role in this tumor with a complex pathogenesis. Further studies, such as RNA sequencing for fusion gene transcripts, may identify other molecular events underlying initiation and progression of this rare neoplasm.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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