Molecular profiling, including *TERT* promoter mutations, of acral lentiginous melanomas

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Acral lentiginous melanoma (ALM) is the less common subtype with singular characterization. *TERT* (human telomerase reverse transcriptase) promoter mutations have being described as recurrent in melanomas and infrequent in ALM, but their real incidence and clinical relevance is unclear. The objectives of this study were to describe the prevalence of *TERT* promoter mutations in ALM, and correlate with the molecular profile of other drive genes and clinical features. Sixty-one samples from 48 patients with ALM were analyzed. After DNA isolation, the mutation profiles of the hotspot region of *BRAF*, *NRAS*, *KIT*, *PDGFRA*, and *TERT* genes were determined by PCR amplification followed by direct Sanger sequencing. *KIT*, *PDGFRA*, and VEGFR2 gene amplification was performed by quantitative PCR. Clinical information such as survival, clinical stage, and Breslow tumor classification were obtained from medical records. *TERT* promoter mutations were found in 9.3% of the cases, *BRAF* in 10.3%, *NRAS* in 7.5%, *KIT* in 20.7%, and *PDGFRA* in 14.8% of ALM. None of the cases showed *KIT*, *PDGFRA*, or *VEGFR2* gene amplification. We found an association between *KIT* mutations and advanced Clark level (IV and V, $P = 0.043$) and *TERT* promoter mutations with low mitotic index. No other significant associations were observed between mutation profile and patients' clinical features nor survival rates. Oncogenic *TERT* promoter mutations are present in a fraction of ALMs. No relevant associations were found between *TERT* mutation status and clinical/molecular features nor survival. Mutations of *KIT* and *PDGFRA* are the most common genetic alterations, and they can be therapeutic targets for these patients. *Melanoma Res* 26:93–99

Melanoma Research 2016, **26**:93–99

Keywords: acral lentiginous, melanoma, molecular profile, TERT promoter mutation

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Received 21 May 2015 **Accepted** 6 November 2015

Introduction

Cutaneous melanoma is the most aggressive skin cancer, with a rising incidence worldwide in the past few decades [1]. Different from the main melanoma subtypes, usually diagnosed in white populations with fair skin and hair, the acral lentiginous melanoma (ALM) is an infrequent counterpart, accounting for less than 2% of all melanoma cases [2,3]. This subtype exhibits unique clinical characteristics, affecting only the palms of the hands, the soles of the feet, and the subungual area [4,5]. In regions with vast contingent of non-white population, such as Asia, Africa, and part of America, ALM is a major concern, as it occurs in populations and skin areas that are not generally at risk for skin cancer, leading frequently to late diagnosis and low survival rates [6–8]. In Brazil, a country with high miscegenated and heterogeneous population with African, European and indigenous heritage, ALM is prevalent in some geographic areas [9–11].

Over the past few years we have witnessed a fast growth in molecular characterization of melanomas, with great emphasis in alterations of cell signaling pathways [12,13]. The mitogen-activated protein kinase pathway has a crucial role in melanoma development and progression [12,14]. *BRAF*, an intracellular serine-threonine kinase, is mutated in ~60% of cutaneous melanomas [12,15,16]. Ninety-five percent of such cases result in a V600E mutation, which involves a valine to glutamic acid substitution at position 600, which leads to a constitutive activation of the mitogen-activated protein kinase pathway [12,14,17]. Mutations are also frequent in another intracellular kinase, particularly in the *NRAS* gene, with a rate of 15–18% of mutated cases [12,14,17]. It has been reported that ALM exhibited a distinct molecular profile when compared with cutaneous melanoma [18]. In the ALM subtype, *BRAF* mutations are uncommon, and mutations were identified in upstream receptor tyrosine kinase, such as *KIT* (15–30%) and *PDGFRA* (6.8%) [12,14,17]. It was also reported that *KIT* and *PDGFRA*

All supplementary digital content is available directly from the corresponding author.

DOI: 10.1097/CMR.0000000000000222

could be altered in ALM by gene amplification mechanisms of their 4p12 locus [18–20]. Besides these two oncogenes, this locus also harbors another important oncogene, *KDR*, also known as *VEGFR2* (vascular endothelial growth factor receptor-2 gene) [21,22]. Importantly, many of the mutated molecules in ALM have emerged for therapeutic targets, such as *BRAF*, *KIT*/*PDGFR*, and *VEGFR2* [23–25].

Recently, another important cancer-related gene, telomerase reverse transcriptase *(TERT*), was described to be mutated in melanomas [26,27]. TERT encodes a subunit of telomerase that, together with other components, elongates the telomere maintaining genomic integrity. Its upregulation has being demonstrated in several human cancers, and the promoter region of the gene is considered the most regulatory element for telomerase expression. *TERT* promoter mutations, namely at positions c. − 124 C > T and c. − 146 C > T, have being reported in up to 50% of cutaneous melanoma [26]. In ALM, the data are scarce, with few studies addressing its biological and clinical impacts [28–30].

In the present study, we intend to determine the frequency of hotspot *TERT* promoter mutations in a series of ALM melanomas. Furthermore, we aimed to assess other major molecular features, such as *BRAF*, *NRAS*, *KIT*, *PDGFRA* mutations, as well as amplification of 4q12 locus (*KIT*/*PDGFRA*/*VEGFR2*), and correlate with *TERT* mutation status and with ALM clinicopathological features.

Materials and methods

Sixty-one formalin-fixed paraffin-embedded tissues from 48 ALM patients were retrieved from the files of the Department of Pathology at Barretos Cancer Hospital. All the patients were diagnosed between 1999 and 2013. Primary tumor samples were obtained from 48 cases. Eleven cases also presented lymph node (LN) samples and in two cases we assessed the distant metastasis. All cases were re-evaluated by a pathologist who confirmed the diagnosis and identified the tumor region for further molecular analysis. The study was conducted according to the national and institutional ethical policies, and it was previously approved by the Local Ethics Committee (CEP-548/2011).

DNA isolation from FFPE tissue

DNA was obtained from formalin-fixed paraffin-embedded tissue sections representative of the tumor lesions, as previously described with some modifications [31]. Briefly, serially 10-μm-thick unstained sections of paraffin blocks were sectioned and one haematoxylin and eosin (H&E) section was used for identification and selection of the tumor area, which was macrodissected into a microfuge tube using a sterile needle (25 G – 0.5 mm, Neolus, Terumo Corporation, Tokyo, Japan). The macrodissected tissue was deparaffinized by a serial wash with xylol and ethanol (100–70–50%) and allowed to air-dry. DNA was extracted using Qiagen's QIAamp DNA Micro Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions and quantified by NanoDrop 2000 (Thermo Scientific, Waltham, Massachusetts, USA). DNA samples were stored at − 20°C until further genetic analysis.

Mutation analysis of *TERT*, *KIT*, *PDGFRA*, and *BRAF* and *NRAS* hotspot regions

The analysis of hotspot mutations of *TERT* promoter regions (contained the sites of c. − 124:C > T and c. − 146:C > T), *KIT* (exons 9, 11, 13, and 17), *PDGFRA* [12,14,18], *BRAF* (exon 15), and *NRAS* (codon12/13, 61), was performed by PCR followed by direct Sanger sequencing, as described previously [28,31–33]. Briefly, using specific pairs of primers (Supplementary Table 1), the target regions were amplified by PCR with an initial denaturation at 95°C for 15 min, followed by 40 cycles of 95°C denaturation for 45 s, specific annealing temperature for 90 s and 72°C elongation for 45 s, and 72°C final elongation for 7 min, in a Verity PCR machine (Applied Biosystems, Carlsbad, California, USA). Amplification of PCR products was confirmed by gel electrophoresis. Sequencing PCR was performed using a Big Dye terminator v3.1 cycle sequencing ready reaction kit (Applied Biosystems) and the ABI PRISM 3500 xL Genetic Analyzer (Applied Biosystems). All mutated cases were confirmed with a second PCR followed by sequencing.

Gene amplification analysis of *KIT*, *PDGFRA*, and *VEGFR2*

KIT, *PDGFRA*, and *VEGFR2* gene amplification was performed by quantitative real-time PCR with LightCycler (Roche Molecular Biochemicals, Mannheim, Germany), using fluorescent hybridization probes and fluorescence resonance energy transfer for the detection of PCR amplification product, as previously described [34,35]. In all assays, appropriate positive and negative controls of 4q12 loci amplification were included [36]. These controls had also been previously assessed by other methodologies, such as array-CGH and CISH [34,36,37].

Statistical analysis

The SPSS 19.0 software (IBM Corp., Armonk, New York, USA) was used for all statistics analyses. To identify associations among clinicopathological characteristics and the molecular alterations, and to compare the prevalence of molecular findings with the previous reports, the Fisher exact test was used. Associations of the same variables with specific cancer survival were analyzed by the Kaplan–Meier method to estimate specific cancer survival, with log-rank testing used to evaluate differences between curves. The *P*-value established for the statistics significance was up to 0.05.

Table 1 Demographic and clinicopathological features of 48 acral lentiginous melanomas

Characteristics	*n* (%)
Sex	
Male	23 (47.9)
Female	25 (52.1)
Age [mean (SD)]	62 (14.8)
Skin	
White	32 (66.7)
Pigmented	15 (31.3)
Not available	1 (2)
Anatomical location	
Hands	3 (6.3)
Feet	45 (93.7)
Breslow depth (mm)	
Up to 1	4 (8.3)
1.1–2	7 (14.6)
2.1–4	16 (33.3)
More than 4	17 (35.4)
Not available	4 (8.3)
Ulceration	
Yes	31 (64.6)
No	10 (20.8)
Not available	7 (14.6)
Mitotic rate (mm^2)	
0	0 (0)
1	11 (22.9)
>1	37 (52.1)
Not available	12 (25)
TNM stage	
I	5 (10.4)
II	22 (45.8)
III	17 (35.4)
IV	4 (8.3)

Results

Clinicopathological features

The characteristics of the patients are shown in Table 1. The age ranged from 26 to 85 years old (mean 62 ± 14.8), and men and women had similar distribution. One-third of the cases presented non-white skin phenotype. Primary lesions were thick and ulcerated in most cases and arose only on the hands in three (6.3%) cases. Localized disease represented half of the cases. Breslow tumor thickness ranged from 0.62 to 20.0 mm, with a median of 4.9 (±4.6). Five-year-specific cancer survival rate for all patients was 51.5%. Follow-up ranged from 2 to 174 months, with a mean of 35.5 (±30.4) and a median of 29.6. At the last evaluation, 20 patients (41.7%) were alive without disease, nine were alive with disease (18.8%), 17 were dead because of melanoma (35.4%), and two were dead because of other causes (4.1%).

Table 3 Exons/codons location of the mutated genes

Mutation	*n* (%)
TERT	
c.−146 C>T	4 (100)
BRAF	
Exon 15 (V600E)	5 (100)
NRAS	
Codon 61	3 (100)
KIT	
Exon 9	1 (16.6)
Exon 11	3 (50)
Exon 13	1 (16.6)
Exon 17	1 (16.6)
PDGFRA	
Exon 12	3 (60)
Exon 14	1 (20)
Exon 18	1 (20)

TERT, telomerase reverse transcriptase.

Molecular profile

Because of the low amount and/or poor DNA quality yield in some samples, we were not able to obtain molecular profiles from all ALM cases. Table 2 summarizes the molecular results. In primary tumors, we observed the presence of 7.0% (3/43) *TERT* mutations, harboring the c.−146 C>T mutation in all cases (Tables 2 and 3). We found a frequency of mutations of 20.7% for *KIT*, 14.8% for *PDGFRA*, 7.5% for *NRAS*, and 10.3% for *BRAF* genes (Table 2). *KIT*, *PDGFRA*, and *VEGFR2* copy numbers were normal in all analyzed cases. One case exhibited two mutations in *PDGFRA*: one at exon 12 and another on exon 14 (Table 3). In the comparison of primary and LN or skin metastasis, we observed a similar molecular status, except in one case that showed *TERT* mutated only in the LN metastasis (Table 2). One case exhibited the same *BRAF* mutation in both primary and LN metastases (Table 2). The co-occurrence of mutations across specimens is demonstrated in Fig. 1. Only *BRAF* mutations were exclusive. *NRAS* was mutated mutually only in one case with *PDGFRA* mutation, and the same was observed with *TERT* and *KIT*; *KIT* and *PDGFRA* presented comutated with two other mutations (Fig. 1).

Clinicopathological and molecular associations

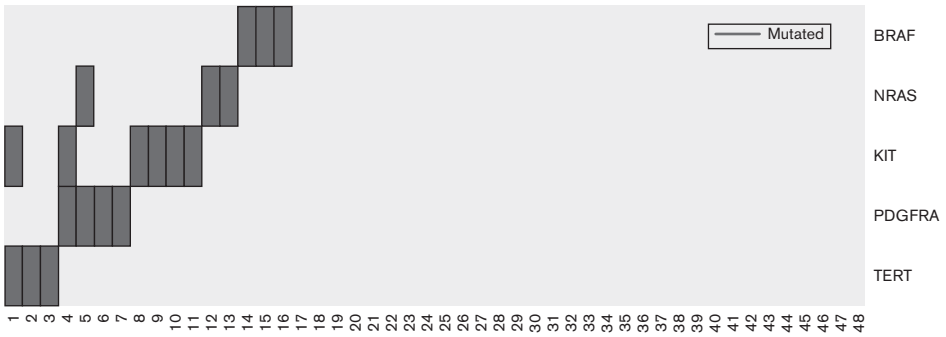
We found a significant association between *KIT* mutation status and higher Clark levels (Table 4). We also observed an association between *TERT* mutations and lower mitosis index (Table 4). There were no significant

Table 2 Molecular profile of acral lentiginous melanomas

	Mutations [*n*/*N* (%)]					Gene amplification [*n*/*N* (%)]		
Patient group	TERT	KIT	PDGFRA	NRAS	BRAF	KIT	PDGFRA	VEGFR2
Primary	3/43 (7.0)	6/29 (20.7)	4/27 (14.8)	3/40 (7.5)	4/39 (10.3)	0/35 (0)	0/36 (0)	0/8 (0)
Lymph node	1/10 (10)	0/10 (0)	0/8 (0)	0/10 (0)	1/11 (9.1)	0/10 (0)	0/10 (0)	0/2 (0)
Metastasis	0/2 (0)	0/2 (0)	0/2 (0)	0/2 (0)	0/2 (0)	0/2 (0)	0/2 (0)	0/0 (0)

TERT, telomerase reverse transcriptase; VEGFR2, vascular endothelial growth factor receptor-2.

Fig. 1



Distribution of *BRAF/NRAS/KIT/PDGFRA* and *TERT* promoter mutations of 48 acral lentiginous melanomas. TERT, telomerase reverse transcriptase.

Table 4 Association of molecular profile with clinicopathological features in acral lentiginous melanomas

	n (%)									
	BRAF		NRAS		KIT		PDGFRA		TERT	
Variables	WT	Mutated	WT	Mutated	WT	Mutated	WT	Mutated	WT	Mutated
Breslow (mm)										
Up to 1	2 (5.7)	0 (0)	3 (8.1)	0 (0)	1 (4.3)	0 (0)	1 (4.3)	0 (0)	2 (5.1)	1 (25)
1.1–2.0	5 (14.3)	1 (25)	6 (16.2)	0 (0)	4 (17.4)	1 (16.7)	4 (17.4)	1 (25)	7 (17.9)	0 (0)
2.1–4.0	12 (34.3)	2 (50)	12 (32.4)	3 (100)	9 (39.1)	3 (50)	10 (43.5)	2 (50)	13 (33.3)	1 (25)
More than 4	13 (37.1)	1 (25)	13 (35.1)	0 (0)	6 (36.1)	2 (33.3)	6 (26.1)	1 (25)	14 (35.9)	2 (50)
Unknown	3 (8.6)	0 (0)	3 (8.1)	0 (0)	3 (13)	0 (0)	2 (8.7)	0 (0)	3 (7.7)	0 (0)
		$P=0.899$		$P=0.248$		$P=0.886$		$P=1.00$		$P=0.594$
Clark										
II	1 (3.1)	0 (0)	1 (2.9)	0 (0)	–	–	–	–	1 (2.8)	0 (0)
III	2 (6.3)	0 (0)	3 (8.8)	0 (0)	3 (15)	0 (0)	2 (9.5)	1 (25)	4 (11.1)	1 (25)
IV	16 (50)	3 (75)	17 (50.0)	3 (100)	14 (70)	2 (33.3)	12 (57.1)	3 (75)	19 (52.8)	1 (25)
V	13 (40.6)	1 (25)	13 (38.2)	0 (0)	3 (15)	4 (66.7)	7 (33.3)	0 (0)	12 (33.3)	2 (50)
		$P=0.736$		$P=0.428$		$P=0.043$		$P=0.400$		$P=0.491$
Ulceration										
Absent	6 (19.4)	0 (0)	6 (18.8)	2 (66.6)	4 (19)	2 (33.3)	5 (22.7)	1 (25)	8 (22.9)	1 (33.3)
Present	25 (80.6)	4 (100)	26 (81.2)	1 (33.3)	17 (81)	4 (66.7)	17 (77.3)	3 (75)	27 (77.1)	2 (66.7)
		$P=0.581$		$P=1.00$		$P=0.588$		$P=1.00$		$P=0.164$
Mitosis										
1	6 (22.2)	2 (66.7)	9 (32.1)	0 (0)	5 (27.8)	2 (33.3)	7 (35)	0 (0)	6 (20)	3 (100)
>1	21 (77.8)	1 (33.3)	19 (67.9)	3 (100)	13 (72.2)	4 (66.7)	13 (65)	3 (100)	24 (80)	0 (0)
		$P=0.166$		$P=0.537$		$P=1.00$		$P=0.526$		$P=$**0.015**
TNM stage										
I	3 (8.6)	0 (0)	4 (10.8)	0 (0)	2 (8.7)	0 (0)	1 (4.3)	1 (25)	4 (10.3)	1 (25)
II	17 (48.6)	2 (50)	18 (48.6)	2 (66.7)	10 (43.5)	4 (66.7)	13 (56.5)	1 (25)	18 (46.2)	1 (25)
III	11 (31.4)	2 (50)	12 (32.4)	1 (33.3)	8 (34.8)	2 (33.3)	7 (30.4)	2 (50)	13 (33.3)	2 (50)
IV	4 (11.4)	0 (0)	3 (8.1)	0 (0)	3 (13)	0 (0)	2 (8.7)	0 (0)	4 (10.3)	0 (0)
		$P=1.00$		$P=1.00$		$P=0.776$		$P=0.440$		$P=0.751$

Fisher's exact test was applied in all cases.
Bold number indicates significant association.
TERT, telomerase reverse transcriptase.

differences in the survival rates according to *TERT*, *BRAF*, *NRAS*, *KIT*, and *PDGFRA* mutation status in the univariate analysis (data not shown).

Discussion

In the present study, we described for the first time the molecular profile of Brazilian acrolentigenous melanomas. We showed that Brazilian patients exhibited a similar profile to the one described in the international literature [12,18–20,24,28–30,38–64] (Table 5).

We found that *TERT* c.−146C>T promoter mutation is present in 9.3% of patients, and this frequency was in concordance with the reported 7.6% (7/92) ($P=0.732$) (Table 5). However, our study represents the larger analysis of the gene hotspot region of *TERT* in this melanoma subtype (Table 5). Previous studies in thyroid cancer and skin melanomas suggested an association of *TERT* mutation with other molecular alterations, such as *BRAF* [28]. In superficial melanomas, thyroid cancer, and gliomas, *TERT* promoter mutations have been also reported to be

Table 5 Summary of molecular features of acral lentiginous melanomas

		Mutations [*n*/*N* (%)]					Gene amplification [*n*/*N* (%)]		
References	Country	TERT	KIT	PDGFRA	NRAS	BRAF	KIT	PDGFRA	VEGFR2
This study	Brazil	4/43 (9.3)	6/29 (20.7)	4/27 (14.8)	3/40 (7.5)	4/39 (10.3)	0/35 (0)	0/36 (0)	0/8 (0)
Heidenreich *et al.* [29]	Spain	4/42 (9.5)	NA	NA	2/38 (5.3)	12/30 (28.6)	NA	NA	NA
Pozzobon *et al.* [38]	Spain	NA	NA	NA	0/10 (0)	2/10 (20)	NA	NA	NA
Liau *et al.* [30]	China	2/32 (6.25)	NA	NA	NA	NA	NA	NA	NA
Dai *et al.* [39]	China	NA	NA	9/132 (9.8)	NA	NA	NA	NA	NA
Vinagre *et al.* [28]	Portugal	1/14 (7.1)	NA	NA	NA	2/14 (14.3)	NA	NA	NA
Hodi *et al.* [20]	Multicenter	NA	2/6 (33.3)	NA	NA	NA	4/4 (100)	NA	NA
Zebary *et al.* [41]	Sweden	NA	13/88 (14.8)	NA	13/88 (14.8)	15/88 (17.0)	NA	NA	NA
Greaves *et al.* [48]	USA	NA	NA	NA	NA	18/111 (16.2)	NA	NA	NA
Dai *et al.* [39]	China	NA	9/39 (23.1)	NA	NA	NA	NA	NA	NA
Lin *et al.* [40]	China	NA	0/20 (0)	NA	NA	NA	NA	NA	NA
Puig-Butillé *et al.* [42]	Spain	NA	NA	NA	3/17 (17.6)	0/17 (0)	NA	NA	NA
Minor *et al.* [24]	USA	NA	3/22 (13.6)	NA	6/22 (27.3)	7/22 (31.8)	6/22 (27.3)	NA	NA
Si *et al.* [47]	China	NA	NA	NA	13/148 (8.8)	23/148 (15.5)	NA	NA	NA
Ashida *et al.* [43]	Japan	NA	4/44 (9.1)	NA	NA	NA	NA	NA	NA
Jakob *et al.* [44]	USA	NA	NA	NA	7/44 (15.9)	6/44 (13.6)	NA	NA	NA
Yun *et al.* [45]	Korea	NA	4/40 (10)	NA	NA	NA	7/20 (35.0)	NA	NA
Kong *et al.* [46]	China	NA	23/193 (11.9)	NA	NA	NA	14/193 (7.3)	NA	NA
Handolias and colleagues [49,50]	Australia	NA	2/6 (33.3)	NA	NA	NA	NA	NA	NA
Terada [51]	Japan	NA	1/2 (50)	0/2 (0)	NA	NA	NA	NA	NA
Handolias and colleagues [49,50]	Australia	NA	1/16 (6.25)	NA	NA	NA	NA	NA	NA
Torres-Cabala *et al.* [52]	USA	NA	5/39 (12.8)	NA	NA	NA	NA	NA	NA
Ashida *et al.* [53]	Japan	NA	2/16 (12.5)	NA	0/22 (0)	3/22 (13.6)	3/16 (18.8)	NA	NA
Curtin *et al.* [54]	USA	NA	NA	0/7 (0)	NA	NA	NA	3/7 (42.9)	NA
Akslen *et al.* [55]	Africa	NA	NA	NA	3/26 (11.5)	1/25 (4.0)	NA	NA	NA
Beadling *et al.* [56]	USA	NA	3/13 (23.1)	NA	1/9 (11.1)	2/12 (16.7)	3/11 (27.3)	NA	NA
Takata *et al.* [57]	Japan	NA	NA	NA	1/6 (16.7)	2/8 (24.05)	NA	NA	NA
Liu *et al.* [59]	Australia	NA	NA	NA	NA	0/6 (0)	NA	NA	NA
Curtin *et al.* [19]	USA	NA	3/28 (10.7)	NA	0/10 (0)	1/10 (10.0)	2/28 (7.1)	NA	NA
Saldanha *et al.* [18]	UK	NA	NA	NA	9/19 (47.4)	2/21 (9.5)	NA	NA	NA
Davison *et al.* [60]	USA	NA	NA	NA	NA	0/3 (0)	NA	NA	NA
Curtin *et al.* [12]	Multicenter	NA	NA	NA	3/35 (8.6)	7/35 (20.0)	NA	NA	NA
Takata *et al.* [58]	Japan	NA	NA	NA	1/28 (3.6)	3/28 (10.8)	NA	NA	NA
Lang and MacKie [61]	UK	NA	NA	NA	NA	2/17 (11.8)	NA	NA	NA
Sasaki *et al.* [62]	Germany	NA	NA	NA	0/15 (0)	5/15 (33.3)	NA	NA	NA
Reifenberger *et al.* [63]	Germany	NA	NA	NA	1/4 (25.0)	2/4 (50.0)	NA	NA	NA
Maldonado *et al.* [64]	USA/Japan	NA	NA	NA	NA	4/30 (13.3)	NA	NA	NA
All the referred studies	_	7/92 (7.6)	75/572 (13.1)	9/145 (6.2)	63/544 (11.6)	119/724 (16.4)	39/294 (13.3)	3/7 (42.9)	NA
Total	_	11/131 (8.4)	81/601 (13.5)	13/168 (7.7)	66/581 (11.4)	123/759 (16.2)	39/329 (11.9)	3/43 (7.0)	0/8 (0)

NA, not available; TERT, telomerase reverse transcriptase; VEGFR2, vascular endothelial growth factor receptor-2.

associated with older patients and worse patient prognosis [65]. In our study, we did not observe such molecular and clinical associations in acrolentigenous melanomas. The association with low mitotic index could be related to better prognosis, but the small number of mutated cases needs further confirmation in a larger series.

Acrolentigenous melanomas are known to harbor a distinct molecular profile of superficial melanomas. Compelling evidence showed the paramount role of *KIT* and *PDGFRA* alterations in the melanoma subtype [66]. In the present study, we found a mutation frequency of 16.7 and 12.5% for *KIT* and *PDGFRA*, respectively. Among *KIT* mutations, 50% were located on exon 11 and the others were distributed among the other exons [9,13, 17], whereas 60% of PDGFRA mutations were located on exon 12 and the others were distributed on exons 14 and 18. These mutation rates are in accordance with previous descriptions of 13.1% (75/572) and 6.2% (9/145) ($P = 0.145$ and 0.108) (Table 5). Some authors have identified *KIT* gene amplification as an alternative mechanism for KIT upregulation in these tumors [18–20]. However, we did not find any case with *KIT* gene amplification using quantitative real-time PCR, a very sensitive and specific methodology, previously optimized by us and reported by our group and other groups [34,36,67,68]. We observed an association between *KIT* mutation and higher Clark staging, indicating that KIT could be associated with a more aggressive disease. The presence of *KIT* and *PDGFRA* alterations, namely mutations, has an important therapeutic impact. It has been shown that some melanoma patients exhibiting *KIT*/*PDGFRA* mutations, rather than gene amplification, can benefit from imatinib-based targeted therapy [69]. Recently, *KIT*/*PDGFRA* mutations have been found as targets for other small inhibitors, such as crenolanib and nilotinib [39,70]. Therefore, we can anticipate that between 15 and 20% of Brazilian acrolentigenous melanoma patients would benefit from these above-mentioned therapeutic modalities.

We also analyzed in this study the frequency of *NRAS* (codon12/13 and 61) and *BRAF* (exon 15) hotspot mutations. We found that *NRAS* was mutated in 6.3% of cases. This frequency was somehow below what is reported worldwide, which is above 11% (63/544) ($P = 0.787$) of cases (Table 5). However, *NRAS* frequency reports range from 0 to 47% (Table 5); therefore, there is not really a consensus in the literature, and this could be because of the distinct sizes of series analyzed in each study, or it could reflect the differences between populations.

With regard to *BRAF*, we observed the presence of 10.6% (all V600E) of mutated cases. This result is in line with the reported (16.4% – 119/724) ($P = 0.639$) (Table 5), where the values range from 0 to 33% of *BRAF* mutated cases. *BRAF* mutations were exclusive events. Besides the low frequency of all mutations, they were mutually present at least in one-third of the cases, except for the *BRAF*, corroborating the high spectrum of melanoma mutations reported.

In conclusion, we have molecularly characterized a series of 48 ALMs. We have shown that less than 10% of patients harbor the recurrent *TERT* promoter mutation, and *KIT* represents the most mutated gene (21%), followed by PDGFRA (15%), in this series. These results could help in the selection of other alternative and potential therapeutic options for Brazilian acral lentiginous melanoma patients.

Acknowledgements

This project was supported by FAPESP – Brazil (2012/4194-1) to Vinicius de Lima Vazquez.

Conflicts of interest

There are no conflicts of interest.

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