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Telomerase Reverse Transcriptase Protein Expression Is More Frequent in Acral Lentiginous Melanoma Than in Other Types of Cutaneous Melanoma

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Context.—Molecularly distinct from cutaneous melanomas arising from sun-exposed sites, acral lentiginous melanomas (ALMs) typically lack ultraviolet-signature mutations, such as telomerase reverse transcriptase (TERT) promoter mutations. Instead, ALMs show a high degree of copy number alterations, often with multiple amplifications of TERT, which are associated with adverse prognosis. The prognostic value of TERT protein expression in acral melanomas, however, is not established.

Objective.—To evaluate the frequency and pattern of TERT immunoreactivity and assess the potential utility of TERT expression as a prognostic indicator in ALMs.

Design.—TERT expression by immunohistochemistry was analyzed in a series of 57 acral and nonacral melanocytic lesions, including 24 primary and 6 metastatic ALMs. Clinical outcome in patients with ALMs by TERT expression was assessed.

Results.—TERT expression was more frequent in ALMs than in nonlentiginous acral melanomas and nonacral cutaneous melanomas, and was absent in acral nevi \( P = .01 \). When present, TERT expression in ALMs was cytoplasmic and more intense than TERT expression in other melanocytic lesions \( P = .05 \) with a higher H-score \( P = .01 \). There was a trend toward decreased overall survival in patients with ALMs with TERT immunoreactivity, but it did not reach statistical significance. Furthermore, no correlation was found between TERT expression and disease-specific survival in patients with ALMs.

Conclusions.—Although TERT protein expression was frequently detected in both primary and metastatic ALMs, TERT immunoreactivity in ALMs did not correlate with survival in our study. Further studies with larger cohorts are needed to elucidate the prognostic value of TERT expression in ALMs.

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Telomeres are regions of repetitive nucleotide sequences located at the ends of chromosomes that play a key role in the maintenance of genomic integrity and stability in cells. In normal nonneoplastic somatic cells, telomeres progressively shorten with successive cell divisions. Repeated truncation of telomeres elicits DNA-damage responses that trigger cellular senescence and chromosomal instability, which increase the likelihood of cellular acquisition of neoplastic transformation. Ironically, once transformed, neoplastic cells require a mechanism to sustain their telomere length so that they can bypass cellular senescence and ultimately gain unlimited replicative capacity; which is a hallmark of cancer. This proliferative immortality in cancer cells is largely achieved by activation or upregulation of telomerase reverse transcriptase (TERT), a catalytic subunit of the enzyme telomerase, which adds nucleotide repeats to the ends of chromosomes, reversing telomere shortening.

The human TERT gene (TERT), which is located on chromosome 5 and encodes TERT, is typically silenced in nearly all cell types except for germ cells, stem cells, lymphoid cells, and basal cells of certain epithelia that regularly undergo cellular division. In fact, mutations in the promoter region of TERT, which lead to increased telomerase activity, are found at high frequency in cutaneous melanomas, particularly in chronically sun-damaged and non–chronically sun-damaged melanomas. These mutations carry an ultraviolet signature (C>T or CC>TT) that results from chronic and intermittent sun exposures, which are associated with chronically sun-damaged (eg, lentigo maligna type) and
non–chronically sun-damaged (eg, superficial spreading and nodular types) melanomas, respectively,\textsuperscript{13,15} and are independently associated with poor prognosis in nonacral cutaneous melanomas (NACMs).\textsuperscript{11,15} In contrast, in primary acral melanoma,\textsuperscript{14,17–21} a rare subtype of cutaneous melanoma that arises in the glabrous skin or the nail apparatus and is associated with a worse prognosis than other NACMs, TERT promoter mutations are less frequent. Rather, TERT copy number gains or amplifications are more common\textsuperscript{22–25} and have been associated with poor outcome in primary acral melanomas.\textsuperscript{19,20} To our knowledge, the prognostic value of TERT expression at a protein level, such as with immunohistochemistry (IHC) in acral melanomas, however, has not been fully investigated to date.

In this study, we evaluated the frequency and pattern of TERT expression by IHC in a series of acral and nonacral melanocytic lesions, with a particular focus on primary and metastatic acral lentiginous melanomas (ALMs), and assessed the utility of TERT protein expression as a potential prognostic indicator in ALMs.

**MATERIALS AND METHODS**

**Case Selection and Review of Clinicopathologic Data**

After obtaining Institutional Review Board approval, we searched our pathology database and identified a total of 57 cases of acral and nonacral melanocytic lesions, including 24 primary ALMs, 6 metastatic ALMs, 10 primary nonlentiginous acral melanomas (NLAMs), 12 primary NACMs, and 5 acral nevi (AN), diagnosed at our institution between 2003 and 2016. Nonlentiginous acral melanoma represents a rare group of acral melanomas without lentiginous growth pattern, showing features comparable with other histologic subtypes of cutaneous melanomas arising in nonacral sites (eg, nodular or superficial spreading subtype). We excluded cases for which residual melanocytic lesions in the formalin-fixed, paraffin-embedded tissue blocks were not sufficient for additional immunohistochemical analysis. Demographic, clinical, and histopathologic parameters and follow-up data for the selected cases were retrieved through review of the final pathology reports and clinical charts. The demographic, clinical, and histopathologic parameters included in our study were sex, median age at diagnosis, race, primary tumor anatomic site, Clark level, Breslow thickness, radial growth phase, vertical growth phase, mitotic rate, ulceration, regression, lymphovascular invasion, perineural invasion, microscopic satellitosis, tumor-infiltrating lymphocytes, associated nevus, predominant cytology; and stage at diagnosis according to the American Joint Committee on Cancer (AJCC) 8th-edition staging manual. In addition, mutation status (eg, mutations in \textit{BRAF}, \textit{KIT}, \textit{NRAS}, etc.), if available, was also recorded.

**Immunohistochemical Analysis of TERT Protein Expression**

A 5-\textmu;m-thick paraffin section was cut from each tissue block of selected cases. The paraffin sections were then tested for TERT protein expression by IHC using an anti-TERT monoclonal rabbit anti-human antibody (clone Y182, 1:100; Abcam, Cambridge, Massachusetts) performed on a Leica Bond autostainer (Leica Biosystems, Buffalo Grove, Illinois) per routine laboratory protocols. External controls using tonsil tissue reacted appropriately, and normal skin, the epidermis, and eccrine glands/ducts in particular (see below for more details) served as internal controls for all immunostained sections.

The pattern of TERT protein expression was recorded as negative, cytoplasmic, nuclear, or Golgi/perinuclear. The intensity of TERT protein expression was recorded as negative (0), weak (1+), moderate (2+), or strong (3+), using the keratinocytes of stratum spinosum and luminal cells of eccrine glands/ducts as reference intensities (designated as 2+ and 3+ intensities, respectively; see Figure 1, A and B) as well as positive internal control. The proportion of TERT protein expression in percentage was also documented. H-score was then calculated using the following formula: [% of positive cells] × [intensity of protein expression].

**Statistical Analysis**

Demographic, clinical, and histopathologic parameters and follow-up time after initial diagnosis were summarized for the selected cases. Categoric variables were summarized by frequencies and percentages and evaluated by Fisher exact test or its generalization, whereas age was summarized by medians and ranges and assessed using Kruskal-Wallis test (3 groups). In addition, associations between TERT expressions and H-score with melanocytic lesions’ group were evaluated by generalizations of the Fisher exact test.

Overall survival (OS) was computed from the time of diagnosis to the last known vital sign. Disease-specific survival (DSS) was computed from the time of diagnosis to the date of death due to melanoma. The Kaplan–Meier method was used to estimate OS and DSS, and differences in survival between groups were assessed using the log-rank test. All statistical analyses were performed using SAS 9.4 for Windows (SAS Institute Inc, Cary, North Carolina). All statistical tests used a significance level of \( P < 0.05 \). No adjustment for multiple testing was made.

**RESULTS**

**Clinicopathologic Characteristics**

The clinicopathologic parameters of each group of melanocytic lesions are summarized in Table 1. Males were more common than females in all groups except for primary NLAMs, which had a male to female ratio of 1:1.5. The median age at diagnosis was similar for 3 groups—primary ALMs, metastatic ALMs, and primary NACMs, all of which had a median age at diagnosis of at least 65 years and older. In contrast, patients with primary NLAMs and AN presented at slightly younger median ages, 56 and 36 years, respectively. Most patients (46 of 57; 81%) were white. However, primary ALMs showed a slightly higher proportion of nonwhite populations (16 whites versus 8 nonwhites) compared with other groups. The foot/toe was the most frequently affected site by acral melanocytic lesions in our study, followed by the hand/finger and the nail bed.

Histologically, nearly all cases of primary ALMs (23 of 24; 96%) had a Clark level of IV or above; the findings were also consistent with those in primary NLAMs and NACMs, 8 of 10 (80%) and 11 of 12 (92%) of which showed invasion into reticular dermis or subcutaneous tissue, respectively. In primary ALMs, 17 of 24 cases (71%) had a Breslow thickness of at least 2.0 mm, whereas 6 of 10 (60%) and 5 of 12 (42%) of primary NLAMs and NACMs, respectively, showed a depth of invasion of 2.0 mm or above. Of note, nearly half of all primary ALMs (11 of 24; 46%) had a Breslow thickness of greater than 4 mm. Most primary melanomas studied were mitotically active, with at least 1 mitosis per square millimeter. Ulceration was significantly more frequent in primary ALMs than in other primary melanomas (\( P = .01 \)). Lymphovascular and perineural invasions were more common in primary acral melanomas than in primary NACMs. Nonbrisk tumor-infiltrating lymphocytes were present in all primary melanomas studied, and the morphology of primary melanomas was predominantly epithelioid. Mutation status was available in 5 cases of primary ALM, 2 cases of metastatic ALM, and 1 case of primary NLAM. Mutations in the \textit{KIT} gene were most common in both primary and metastatic ALMs combined. Overall, the stage at diagnosis was higher in primary acral melanomas than in primary NACMs.
Figure 1. Telomerase reverse transcriptase (TERT) expression in normal skin and acral lentiginous melanoma (ALM). The stratum spinosum layer of the epidermis (A) showed cytoplasmic TERT expression, which was designated as 2+ intensity (red arrows), whereas the luminal cells of eccrine glands/ducts (B) exhibited TERT in a greater intensity, which was designated as 3+ (red arrows). Both served as positive internal controls. In our series of ALMs, cytoplasmic expression of TERT within the tumor cells was frequently diffuse and homogeneous with a moderate staining intensity (2+), leading to a high (>100) H-score overall (C and D). Note that the intensity of TERT expression in this example of ALM is similar to that in the adjacent stratum spinosum layer, corresponding to 2+ intensity (TERT, original magnifications ×400 [A, B, and D]; hematoxylin-eosin, original magnification ×200 [C]).
Table 1. Demographic, Clinical, and Histopathologic Characteristics by Lesion Type

| Characteristic                  | Primary Acral Lentiginous Melanoma (n = 24) | Metastatic Acral Lentiginous Melanoma (n = 6) | Nonlentiginous Acral Melanomaa (n = 10) | Primary Cutaneous Melanoma, Nonacral (n = 12) | Acral Nevus (n = 5) |
|--------------------------------|---------------------------------------------|----------------------------------------------|----------------------------------------|-----------------------------------------------|------------------|
| **Sex**                        |                                             |                                              |                                        |                                               |                  |
| Male                           | 15 (63)                                     | 4 (67)                                       | 4 (40)                                 | 7 (58)                                        | 3 (60)           |
| Female                         | 9 (38)                                      | 2 (33)                                       | 6 (60)                                 | 5 (42)                                        | 2 (40)           |
| **Median age at diagnosis (range), y** | 71 (38–89)                              | 72 (31–82)                                  | 56 (2–89)                              | 66 (14–93)                                    | 36 (34–62)       |
| **Race/ethnicity**             |                                             |                                              |                                        |                                               |                  |
| White                          | 16 (67)                                     | 5 (83)                                       | 8 (80)                                 | 12 (100)                                      | 5 (100)          |
| Hispanic                       | 7 (29)                                      | 0                                            | 0                                      | 0                                             | 0                |
| African American               | 0                                           | 1 (17)                                       | 1 (10)                                 | 0                                             | 0                |
| Asian                          | 1 (4)                                       | 0                                            | 1 (10)                                 | 0                                             | 0                |
| **Primary tumor site**         |                                             |                                              |                                        |                                               |                  |
| Hand/finger                    | 2 (8)                                       | 0                                            | 1 (10)                                 | 1 (8)                                         | 0                |
| Foot/toe                       | 21 (88)                                     | 6 (100)                                      | 9 (90)                                 | 0                                             | 5 (100)          |
| Nailbed                        | 1 (4)                                       | 0                                            | 0                                      | 0                                             | 0                |
| Other                          | 0                                           | 0                                            | 0                                      | 11 (92)                                       | 0                |
| **Clark level**                |                                             |                                              |                                        |                                               |                  |
| II                             | 1 (4)                                       | —                                            | 1 (10)                                 | 0                                             | —                |
| III                            | 0                                           | —                                            | 1 (10)                                 | 1 (8)                                         | —                |
| IV                             | 16 (67)                                     | —                                            | 5 (50)                                 | 8 (67)                                        | —                |
| V                              | 7 (29)                                      | —                                            | 3 (30)                                 | 3 (25)                                        | —                |
| **Breslow thickness**          |                                             |                                              |                                        |                                               |                  |
| ≤1 mm                          | 0                                           | —                                            | 1 (10)                                 | 3 (25)                                        | —                |
| 1.01–2 mm                      | 7 (29)                                      | —                                            | 3 (30)                                 | 4 (33)                                        | —                |
| 2.01–4 mm                      | 6 (25)                                      | —                                            | 4 (40)                                 | 3 (25)                                        | —                |
| >4 mm                          | 11 (46)                                     | —                                            | 2 (20)                                 | 2 (17)                                        | —                |
| **Radial growth phase**        |                                             |                                              |                                        |                                               |                  |
| Present                        | 15 (63)                                     | —                                            | 3 (30)                                 | 9 (75)                                        | —                |
| Not identified                 | 9 (38)                                      | —                                            | 7 (70)                                 | 3 (25)                                        | —                |
| **Vertical growth phase**      |                                             |                                              |                                        |                                               |                  |
| Present                        | 24 (100)                                    | —                                            | 8 (80)                                 | 12 (100)                                      | —                |
| Not identified                 | 0                                           | —                                            | 2 (20)                                 | —                                             | —                |
| **Mitotic rate**               |                                             |                                              |                                        |                                               |                  |
| <1 per mm²                     | 0                                           | —                                            | 2 (20)                                 | 2 (17)                                        | —                |
| 1–4 per mm²                    | 14 (58)                                     | —                                            | 5 (50)                                 | 9 (75)                                        | —                |
| 5–9 per mm²                    | 5 (21)                                      | —                                            | 0                                      | 1 (8)                                         | —                |
| 10–20 per mm²                  | 3 (13)                                      | —                                            | 3 (30)                                 | 0                                             | —                |
| >20 per mm²                    | 2 (8)                                       | —                                            | 0                                      | 0                                             | —                |
| **Ulceration**                 |                                             |                                              |                                        |                                               |                  |
| Present                        | 14 (58)                                     | —                                            | 2 (20)                                 | 2 (17)                                        | —                |
| Not identified                 | 10 (42)                                     | —                                            | 8 (80)                                 | 10 (83)                                       | —                |
| **Regression**                 |                                             |                                              |                                        |                                               |                  |
| Present                        | 3 (13)                                      | —                                            | 1 (10)                                 | 2 (17)                                        | —                |
| Not identified                 | 21 (88)                                     | —                                            | 9 (90)                                 | 10 (83)                                       | —                |
| **Lymphovascular invasion**    |                                             |                                              |                                        |                                               |                  |
| Present                        | 4 (17)                                      | —                                            | 3 (30)                                 | 0                                             | —                |
| Not identified                 | 20 (83)                                     | —                                            | 7 (70)                                 | 12 (100)                                      | —                |
| **Perineural invasion**        |                                             |                                              |                                        |                                               |                  |
| Present                        | 9 (38)                                      | —                                            | 2 (20)                                 | 1 (8)                                         | —                |
| Not identified                 | 15 (63)                                     | —                                            | 8 (80)                                 | 11 (92)                                       | —                |
| **Microscopic satellitosis**   |                                             |                                              |                                        |                                               |                  |
| Present                        | 3 (13)                                      | —                                            | 1 (10)                                 | 0                                             | —                |
| Not identified                 | 21 (88)                                     | —                                            | 9 (90)                                 | 12 (100)                                      | —                |
Frequency and Intensity of TERT Protein Expression and H-Score

The proportion and intensity of TERT expressions and corresponding H-scores in each group of melanocytic lesions are summarized in Table 2. The pattern of TERT expression was cytoplasmic in all positive cases, similar to those seen in our internal and external controls. TERT expression was present in 16 of 24 primary ALMs (67%; Figure 1, C and D), 4 of 10 primary NLAMs (40%; Figure 2, A and B), and 7 of 12 primary NACMs (58%; Figure 2, C and D). Notably, all metastatic ALMs were positive for TERT, whereas all AN, including 1 case with severe architectural disorder, lacked immunoreactivity for TERT. Overall, the frequency of TERT expression in primary ALMs with respect to other groups of melanocytic lesions was statistically significant ($P = .01$). Moderate-to-strong (≥2+) TERT expression was more frequently seen in primary and metastatic ALMs than in primary NLAMs, and half of primary NACMs showed a moderate-to-strong expression of TERT ($P = .05$). Among the melanomas expressing TERT, 14 of 24 (58%) and 3 of 6 (50%) cases of primary and metastatic ALMs, respectively, had a higher H-score (>100), whereas 4 of 12 cases of primary NACMs (33%) and 2 of 10 cases of NLAMs (20%) fell into the same H-score category ($P = .01$).

OS and DSS With Respect to TERT Protein Expression in Patients With ALMs

The OS and DSS in patients with primary and metastatic ALMs with respect to the presence or absence, intensity, and H-score of TERT expression are summarized in Table 3. There was a trend toward decreased OS in patients with primary and metastatic ALMs expressing TERT in a moderate to strong intensity with a higher H-score (>100; Figure 3, A through C); however, this did not reach statistical significance ($P = .16$ to .36). Furthermore, there was no significant correlation between DSS and TERT expression, TERT expression intensity, and H-score in patients with primary and metastatic ALMs ($P = .19$ to .44; Figure 3, D through F).

DISCUSSION

In this study, we evaluated the frequency and pattern of TERT expression and assessed the potential prognostic value of TERT expression by IHC in a series of primary and metastatic ALMs. We found TERT expression in 67% of primary ALMs and all metastatic ALMs but no AN, including 1 case with severe architectural disorder. In contrast, the frequency of TERT expression was lower in primary NACMs than in primary ALMs, with 58% of primary NACMs being positive for TERT immunohistochemically. This is slightly higher than the reported
frequency of 44% demonstrated recently by Hugdahl et al.26 In their study, TERT protein expression was seen in 44% of primary cutaneous melanomas (all nodular types; n = 108) and 16% of metastatic melanomas (n = 11). Our small sample size of primary NACMs (n = 12) may have contributed to the higher rate of TERT expression, although the reported likelihood of TERT expression in cutaneous melanomas appears to be highly variable across studies. For instance, Populo et al.13 previously reported that 98% of cutaneous melanomas were positive for TERT by IHC in their series of 116 cases of cutaneous melanomas, 22 of which were ALMs. However, these authors did not report the exact proportion of ALMs expressing TERT, making a direct comparison with our data challenging. More recently, de Unamuno Bustos et al.27 reported that TERT protein expression was seen in all melanocytic lesions, including nevi as well as primary and metastatic cutaneous melanomas, 3 of which were ALMs. Nonetheless, in our study, there was a significant difference in the rate of TERT expression between primary ALMs and other melanocytic lesions, including primary NACMs and AN (P = .01). Our finding with the latter group AN, with the complete absence of TERT expression, in particular may serve as a useful ancillary tool for distinguishing AN from primary ALMs, especially if ambiguous features raising concern for possible melanoma are present in AN. Further validation with a larger cohort is needed, however, to confirm this impression, because a few prior studies23,26 have demonstrated the presence of TERT immunoreactivity in AN, albeit weaker in intensity than those in their malignant counterparts.

The expression pattern of TERT in our series was cytoplasmic, similar to the findings seen in a few prior studies.23,26 In a study by Hugdahl et al.26 TERT expression in their cohort of primary and metastatic NACMs was consistently cytoplasmic and generally homogeneous without significant heterogeneity in staining intensity. Populo et al.13 and Diaz et al.23 reported cytoplasmic and nuclear expression of TERT in ALMs. Intriguingly, in a study by Diaz et al.23 TERT expression in ALMs was both nuclear and cytoplasmic, whereas all AN exhibited cytoplasmic expression of TERT, unlike in our study wherein all AN, including 1 with severe architectural disorder, lacked immunoreactivity for TERT. De Unamuno Bustos et al.27 reported heterogeneous cytoplasmic expression of TERT with a stronger staining intensity in cutaneous melanomas, whereas weak, homogeneous cytoplasmic immunoreactivity for TERT was seen in their series of nevi. However, our findings regarding TERT expression were incongruent with the findings reported in another study, by Kohli et al.28 In their study, TERT expression was either nucleolar or “non-nucleolar” (defined by the authors as protein expression in subnuclear foci or diffuse nuclear positivity), with the so-called nonnucleolar pattern showing a positive trend with increasing frequency of immunoreactivity, as melanocytic lesions progressed from benign nevi to metastatic melanoma.

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**Table 2. Telomerase Reverse Transcriptase (TERT) Expression by Lesion Type**

| Characteristic          | Primary Acral Lentiginous Melanoma (n = 24) | Metastatic Acral Lentiginous Melanoma (n = 6) | Nonlentiginous Acral Melanoma (n = 10) | Primary Cutaneous Melanoma, Nonacral (n = 12) | Acral Nevus (n = 5) | P Value |
|-------------------------|--------------------------------------------|----------------------------------------------|---------------------------------------|-----------------------------------------------|-------------------|---------|
| TERT expression         |                                            |                                              |                                       |                                               |                   | .01     |
| Positive                | 16 (67)                                    | 6 (100)                                      | 4 (40)                                | 7 (58)                                        | 0                 |         |
| Negative                | 8 (33)                                     | 0                                            | 6 (60)                                | 5 (42)                                        | 5 (100)           |         |
| TERT expression intensity |                                           |                                              |                                       |                                               |                   | .05     |
| Absent/weak             | 10 (42)                                    | 2 (33)                                       | 8 (80)                                | 6 (50)                                        | 5 (100)           |         |
| Moderate/strong          | 14 (58)                                    | 4 (67)                                       | 2 (20)                                | 6 (50)                                        | 0                 |         |
| H-scorea                 | 0                                          | 8 (33)                                       | 6 (60)                                | 5 (42)                                        | 5 (100)           | .01     |
| >0-100                   | 2 (8)                                      | 3 (50)                                       | 2 (20)                                | 3 (25)                                        | 0                 |         |
| >100                     | 14 (58)                                    | 3 (50)                                       | 2 (20)                                | 4 (33)                                        | 0                 |         |

*Calculated as (% of positive cells) × (intensity of protein expression). Bolded P values indicate statistically significant P value ≤ .05.

**Table 3. Overall Survival and Disease-Specific Survival in Patients With Primary and Metastatic Acral Lentiginous Melanoma by Telomerase Reverse Transcriptase (TERT) Expression**

| Rates, Total:NOD 3-y:5-y:10-y:EOA, % | P Value |
|--------------------------------------|---------|
| Overall survival                     |         |
| TERT expression                      |         |
| Negative                             | 8:2     | 100:57:57:57 | .16 |
| Positive                             | 22:12   | 70:40:20:0   |     |
| TERT intensity                       |         |
| Absent/weak                          | 12:4    | 92:41:41:41  | .31 |
| Moderate/strong                      | 18:10   | 70:43:22:0   |     |
| H-score                              | 0       | 8:2          | 100:57:57:57 | .36 |
| >0 to 100                            | 5:2     | 80:0:0       |     |
| >100                                 | 17:10   | 68:43:21:0   |     |
| Disease-specific survival            |         |
| TERT expression                      |         |
| Negative                             | 8:0     | 100:100:100:100 | .19 |
| Positive                             | 22:4    | 80:80:80:80  |     |
| TERT intensity                       |         |
| Absent/weak                          | 12:1    | 92:92:92:92  | .44 |
| Moderate/strong                      | 18:3    | 81:81:81:81  |     |
| H-score                              | 0       | 8:0          | 100:100:100:100 | .42 |
| >0 to 100                            | 5:1     | 80:80:80:80  |     |
| >100                                 | 17:3    | 80:80:80:80  |     |

Abbreviations: EOA, end of assessment; NOD, number of deaths.
Telomerase reverse transcriptase (TERT) expression in nonlentiginous acral melanoma (NLAM) and nonacral cutaneous melanoma (NACM). Rarely, acral melanomas exhibit nonlentiginous growth pattern histologically. This particular example of NLAM was nodular subtype without a radial growth phase (A), exhibiting 1+ TERT staining intensity (B). The intensity of TERT expression and proportion of TERT-positive cells could also vary in cutaneous melanomas. Note that in this example of NACM (C) the atypical junctional melanocytic nests appear to exhibit stronger staining intensity than the invasive component within the dermis, partly due to prominent melanin pigments (C, red arrows); one should be cautious not to misinterpret these darkly stained pigments as "nuclear" or "Golgi/perinuclear" TERT expression. Rarely, TERT expression varied with the cytomorphology of melanoma cells. In one case of NACMs, the tumor cells with epithelioid morphology (D, red arrows) exhibited 1+ TERT staining intensity, whereas the spindled cell components (D, yellow arrows) lacked TERT expression (hematoxylin-eosin, original magnification ×200 [A]; TERT, original magnifications ×200 [B and D] and ×400 [C]).
A possible explanation for this discrepancy could be the analytic differences between the studies, including clones of anti-TERT antibodies and methods of antigen retrieval.

It is noteworthy that we also observed the presence of cytoplasmic TERT expression in normal epidermis and eccrine glands, similar to the findings seen in a prior study by de Unamuno Bustos et al.\(^27\) In addition, we further documented that immunoreactivity for TERT protein was specifically localized in the stratum spinosum layer of the epidermis (Figure 1, A) and luminal cells of eccrine glands/ducts (Figure 1, B), findings that have not been described previously in detail. It is unclear at this point as to why the stratum spinosum layer, not the regenerative basal layer of the epidermis (as previously witnessed in cultured basal keratinocytes that exhibited higher telomerase enzymatic activity), consistently showed TERT protein expression in our study. Nonetheless, these findings help establish the appropriate internal controls for TERT immunohistochemical testing, at least in the skin. Given the lack of a universally standardized anti-TERT antibody clone for immunohistochemical analysis and the seemingly conflicting expression patterns reported to date,\(^13,23,26–28\) as outlined above, our findings with 2 different tissue types on the same plane of section that show conspicuously distinct staining intensities will be of value to proper assessment and potential validation of the immunohistochemical assay for future studies investigating cutaneous lesions.

In our study, compared with TERT expression in NACMs and NLAMs, TERT expression in ALMs (both primary and metastatic) more commonly exhibited moderate-to-strong (\(^{\pm}2\)) intensity (\(P = .05\)) and higher H-score (\(>100\); \(P = .01\)). Despite the higher frequency and stronger expression with a higher H-score, TERT protein expression by IHC in patients with ALMs did not correlate with OS and DSS in our cohort. Given that there was a trend toward decreased OS in our patients with primary and metastatic ALMs with stronger intensity of TERT expression and an H-score greater than 100, we attempted to increase the cutoff value for a “high” H-score to 150 or 200 and reassessed OS and DSS; however, the relationship between H-score and survival still did not reach statistical significance (unpublished data). Our results are similar to the findings of a few prior studies that showed no correlation between TERT

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Figure 3. Overall and disease-specific survivals with respect to telomerase reverse transcriptase (TERT) expression in patients with primary and metastatic acral lentiginous melanoma (ALM). There was a trend toward decreased overall survival in patients with ALMs expressing TERT in a moderate-to-strong staining intensity with a higher H-score (\(>100\); A through C), although this did not reach statistical significance (\(P > .05\)). In addition, TERT expression, TERT intensity, and H-score in primary and metastatic ALMs did not correlate with disease-specific survival (\(P > .05\); D through F).

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\(A\), \(B\), \(C\), \(D\), \(E\), \(F\)
protein expression and TERT promoter mutation or amplification status, as well as patient survival in cutaneous melanomas. In a study by Hugdahl et al., although TERT protein expression did not correlate with mutation status, it was found to be associated with reduced patient survival. More recently, de Unamuno Bustos et al. demonstrated an association between heterogeneous expression pattern of TERT and aggressive phenotype as well as TERT promoter mutation status in cutaneous melanomas. Both of these studies, however, almost exclusively assessed NACMs and included few cases of ALM. Thus, further carefully designed studies with a larger cohort of ALMs are needed to more accurately assess the utility of TERT immunoreactivity as a prognostic indicator. Given that TERT promoter mutations are uncommon in ALMs, correlations between TERT immunohistochemical expression and TERT copy number gain or amplification status need to be further investigated to better evaluate the utility of TERT expression as a potential surrogate marker for the aforementioned molecular alterations (eg, TERT amplifications) known to be associated with adverse prognosis.

In conclusion, our study demonstrated that unlike TERT promoter mutations, TERT protein expression was frequently detected in both primary and metastatic ALMs. In addition, to the best of our knowledge, our study is the first to demonstrate differences in TERT immunohistochemical expression between ALMs and NLAMs, which have never been separately studied but rather have been grouped together (ie, as “acral melanomas”) without histologic distinction. Lastly, in our study, although TERT expression was more frequent and of stronger intensity in ALMs than in other types of melanocytic lesions, with higher overall H-scores, TERT immunoreactivity in ALMs did not correlate with survival. For future studies, it would be interesting to see if these results would be replicated in a larger cohort of patients with ALMs and also if TERT expression would correlate with TERT copy number gains or amplifications, which are more common than TERT promoter mutations in acral melanomas. In recent years, many attempts have been made to develop therapeutic agents targeting TERT given its critical role in tumorigenesis. These efforts led to the development of several telomerase-based therapeutic strategies in various forms, some of which showed anticancer efficacy in solid tumors, including melanoma. These antitelomerase therapies, albeit still in the preliminary stages of development, may provide survival benefit for patients with ALM in the future.

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References
1. Gunes C, Rudolph KL. The role of telomeres in stem cells and cancer. Cell. 2013;152(3):390–393.
2. Jafri MA, Ansari SA, Alghathi MH, Shay JW. Roles of telomeres and telomerase in cancer, and advances in telomerase-targeted therapies. Gene. 2016;571(1):69.
3. Jackson SP, Bartek J. The DNA-damage response in human biology and disease. Nature. 2009;461(7267):1071–1078.
4. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell. 2000;100(1):57–70.
5. Harle-Bachor C, Boukamp P. Telomerase activity in the regenerative basal layer of the epidermis in human skin and in immortal and carcinoma-derived skin keratinocytes. Proc Natl Acad Sci U S A. 1996;93(13):6476–6481.
6. Shyu JW, Weight WE. Senescence and immortalization: role of telomeres and telomerase. Carcinogenesis. 2005;26(5):867–874.
7. Norrback KE, Roos G. Telomeres and telomerase in normal and malignant haematopoietic cells. Eur J Cancer. 1997;33(5):774–780.
8. Barsov EV. Telomerase and primary T cells: biology and immunomodulation for adoptive immunotherapy. Immunotherapy. 2011;3(3):407–421.
9. Bougil S, Renaud S, Braunschweig R, et al. PAX5 activates the transcription of the human telomerase reverse transcriptase gene in B cells. J Pathol. 2010;220(1):87–96.
10. Parness CN, Jezzard S, Silver A, Mackie R, McGregor JM, Newbold RF. Telomerase activity in melanoma and non-melanoma skin cancer. Br J Cancer. 1999;79(1):47–53.
11. Horn S, Figl A, Rachakonda PS, et al. TERT promoter mutations in familial and sporadic melanoma. Science. 2013;339(6212):959–961.
12. Huang FW, Hodis E, Xu MJ, Kryukov GV, Chin L, Garraway LA. Highly recurrent TERT promoter mutations in human melanoma. Science. 2013;339(6212):957–959.
13. Populo H, Boaventura P, Vinagre J, et al. TERT promoter mutations in skin cancer: the effects of sun exposure and X-irradiation. J Investig Dermatol. 2014;134(8):2251–2257.
14. Grosvang KG, Murali R, Puig-Butille JA, et al. TERT promoter mutation status as an independent prognostic factor in cutaneous melanoma. J Natl Cancer Inst. 2014;106(9):djv246.
15. Curtin JA, Fridlyand J, Kageshita T, et al. Distinct sets of genetic alterations in melanoma. N Engl J Med. 2005;353(20):2135–2147.
16. Bastian BC. The molecular pathology of melanoma: an integrated taxonomy of melanocytic neoplasia. Annu Rev Pathol. 2014;9:239–271.
17. Heslenreich N, Nagore E, Rachakonda PS, et al. Telomerase reverse transcriptase promoter mutations in primary cutaneous melanoma. Nat Commun. 2014;5:3401.
18. Roh MR, Park KH, Chung KY, Shin SJ, Rha SY, Tsao H. Telomerase reverse transcriptase (TERT) promoter mutations in Korean melanoma patients. Am J Cancer Res. 2017;7(3):134–138.
19. Yeh I, Jorgenson E, Shen L, et al. Targeted genomic profiling of acral melanoma. J Natl Cancer Inst. 2019;111(10):1068–1077.
20. Liu JY, Tsai JH, Jeng YM, Chu CY, Kuo KT, Liang CW. TERT promoter mutation is uncommon in acral lentiginous melanoma. J Cutan Pathol. 2014;41(6):504–508.
21. Vazquez Vde L, Vicente AL, Carlino A, et al. Molecular profiling, including TERT promoter mutations, of acral lentiginous melanomas. Melanoma Res. 2016;26(2):92–99.
22. Motaparthi K, Kim J, Andea AA, et al. TERT and TERT promoter mutations in melanocytic neoplasms: Current concepts in pathogenesis, diagnosis, and prognosis (published online ahead of print March 23, 2020). J Cutan Pathol. 2020. doi: 10.1111/cup.13691.
23. Diaz A, Puig-Butille JA, Valera A, et al. TERT and AURKA gene copy number gains enhance the detection of acral lentiginous melanomas by fluorescence in situ hybridization. J Med Diag. 2014;16(2):198–206.
24. Yu S, Xu T, Dai J, et al. TERT copy gain predicts the outcome of high-dose interferon α-2b therapy in acral melanoma. Oncol Targets Ther. 2018;11:4097–4104.
25. Puig-Butille JA, Badenças C, Ogbah Z, et al. Genetic alterations in RAS-regulated pathway in acral lentiginous melanoma. Exp Dermatol. 2013;22(2):148–150.
26. Hugdahl E, Kalvenes MB, Mannevig M, Ladstein RG, Akslen LA. Prognostic impact and concordance of TERT promoter mutation and protein expression in matched primary and metastatic cutaneous melanoma. Br J Cancer. 2018;118(1):98–105.
27. de Unamuno Bustos B, Sahugullio Torralba A, Mules Poveda P, et al. Telomerase expression in a series of melanocytic neoplasms. Actas Dermosifiliogr. 2019;110(3):212–219.
28. Kohlh JS, Mir H, Wasif A, et al. ETS1, nucleolar and non-nucleolar TERT expression in nevus to melanoma progression. Oncotarget. 2017;8(61):104408–104417.
29. Reyes-Uribe P, Adrianzen-Ruesta MP, Deng Z, et al. Exploiting TERT dependency as a therapeutic strategy for NRAS-mutant melanoma. Oncogene. 2018;37(30):4058–4072.
30. Mizukoshi E, Kaneko S. Telomerase-targeted cancer immunotherapy. Int J Mol Sci. 2019;20(8):1823.
31. Liu JP, Chen W, Schweizer AP, Li H. Telomerase in cancer immunotherapy. Biochim Biophys Acta. 2010;1805(1):35–42.