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REVIEW

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TERT and *TERT* promoter in melanocytic neoplasms: Current concepts in pathogenesis, diagnosis, and prognosis

Kiran Motaparthi MD[1] | Jinah Kim MD, PhD[2] | Aleodor A. Andea MD[3,4] | Tricia A. Missall MD, PhD[1] | Roberto A. Novoa MD[5,6] | Claudia I. Vidal MD, PhD[7] | Maxwell A. Fung MD[8] | Patrick O. Emanuel MBChB, FRCPA[9,10]

[1]Department of Dermatology, University of Florida College of Medicine, Gainesville, Florida

[2]Palo Alto Medical Foundation, Palo Alto, California

[3]Department of Dermatology, University of Michigan Medical Center, Ann Arbor, Michigan

[4]Department of Pathology, University of Michigan Medical Center, Ann Arbor, Michigan

[5]Department of Dermatology, Stanford University School of Medicine, Stanford, California

[6]Department of Pathology, Stanford University School of Medicine, Stanford, California

[7]Dermatology Center of Southern Indiana, Bloomington, Indiana

[8]Department of Dermatology, University of California, Davis, California

[9]Laboratorio Recavarren Emanuel, Clínica Ricardo Palma, Lima, Peru

[10]IGENZ Molecular Laboratory, Auckland, New Zealand

Correspondence
Patrick O. Emanuel, Laboratorio Recavarren Emanuel, Clínica Ricardo Palma, Lima, Peru.
Email: paddyemanuel@gmail.com

Abstract

Background and objective: Located on chromosome locus 5p15.33, telomerase reverse transcriptase (*TERT* or h*TERT*) encodes the catalytic subunit of telomerase which permits lengthening and preservation of telomeres following mitosis. Mutations in *TERT* promoter (*TERT*-p) upregulate expression of *TERT*, allowing survival of malignant cells and tumor progression in wide variety of malignancies including melanoma. The objective of this review is to examine the roles of *TERT* and *TERT-p* in the pathogenesis, diagnosis, and prognostication of cutaneous melanoma.

Methods: All studies of *TERT* or *TERT-p* in cutaneous melanocytic neoplasms with the following inclusion criteria were reviewed: publication date between 2010 and 2019, English language, and series of ≥3 cases were reviewed for evidence supporting the role of *TERT* in pathogenesis, diagnosis, and prognosis. Studies with <3 cases or focused primarily on mucosal or uveal melanocytic tumors were excluded.

Results and conclusion: *TERT*-p mutations are frequent in chronic and non-chronic sun damage melanoma and correlate with adverse prognosis, inform pathogenesis,

Abbreviations: AJCC, American Joint Committee on Cancer; ALM, acral lentiginous melanoma; AM, acral melanoma; AN, acral nevus; CSD, chronic sun damage; DM, desmoplastic melanoma; DNA, deoxyribonucleic acid; ETS, E26 transformation specific; FISH, fluorescence in situ hybridization; GCN, giant congenital nevus; IHC, immunohistochemistry; ISH, in situ hybridization; LM, lentigo maligna; LMM, LM melanoma; MAPK, mitogen-activated protein kinase; MDM, mixed desmoplastic melanoma; MIS, melanoma in situ; MM, metastatic melanoma; MPM, multiple primary melanoma; MUP, melanoma of unknown primary; NGS, next-generation sequencing; NM, nevoid melanoma; PCR, polymerase chain reaction; PDM, pure desmoplastic melanoma; PHH3, phosphohistone H3; RT, real time; SSM, superficial spreading melanoma; TCF, ternary complex subfamily; *TERT*-p, *TERT* promoter; WT, wild type; Hotspot mutations, c. − 124:C > T (C228T) and c. −146:C > T (C250T); rs2853669 polymorphism, −245 T > C polymorphism; UV signature:, C > T or CC > TT; Tandem mutations, CC > TT at positions −124/−125 or −138/−139 bp.

and may provide diagnostic support. While *TERT*-p mutations are uncommon in acral melanoma, *TERT* copy number gains and gene amplification predict reduced survival. Among atypical spitzoid neoplasms, *TERT*-p mutations identify biologically aggressive tumors and support the diagnosis of spitzoid melanoma. *TERT*-p methylation may have prognostic value in pediatric conventional melanoma and drive tumorigenesis in melanoma arising within congenital nevi. Finally, *TERT*-p mutations may aid in the differentiation of recurrent nevi from recurrent melanoma.

KEYWORDS

melanocytic, melanoma, molecular, *TERT*, *TERT* promoter

1 | INTRODUCTION

In the pedigree of a melanoma-prone family, a unique germline T > G mutation was identified within the telomerase reverse transcriptase promoter (*TERT*-p), located 57 bp upstream from the ATG translation start site of *TERT* (5p15.33). This mutation creates a binding motif for E26 transformation-specific (ETS) transcription factors and ternary complex factors (TCFs) that increase *TERT* expression.[1,2] ETS transcription factors are expressed in early nevogenesis, prior to extranucleolar telomerase localization and activity.[3] Horn et al initially sequenced *TERT*-p in sporadic primary and metastatic melanomas. While these tumors did not harbor the familial mutation, recurrent and mutually exclusive ultraviolet (UV)-signature (C > T or CC > TT) mutations within a 49 bp region −100 bp upstream from the ATG start site were identified. Similar to the germline mutation, these UV-signature mutations created ETS/TCF binding motifs. The two most common mutations ("hotspot") were located at −124 bp (C > T) and −146 bp (C > T). Two tandem mutations (CC > TT) at −124/−125 and −138/−139 were also identified, and the latter may result from a single mutation at −138 because the base mutation at −139 may occur as a polymorphism (rs35550267).[1,2]

Expression of TERT by RT-PCR demonstrated overlapping profiles in dysplastic nevi and melanoma, suggesting the role of *TERT* in precursor lesions.[4] Targeted sequencing of 293 cancer genes was performed in 37 primary melanomas with adjacent precursor lesions by Shain et al. In precursor lesions, including intermediate melanocytic neoplasms and melanomas in situ (MIS), 77% harbored *TERT*-p mutations. In contrast, all unequivocally benign areas harbored *BRAF* V600E mutations only. In contrast, biallelic inactivation of *CDKN2A* and copy-number alterations were later events only observed in invasive melanoma, while *PTEN* and *TP53* mutations were limited to advanced tumors. Thus, *TERT*-p mutations are selected at an early age of tumorigenesis and represent the earliest secondary alterations in intermediate neoplasms and MIS. Benign precursors with alterations within the mitogen-activated protein kinase (MAPK) pathway may persist through *TERT*-p mutations and then acquire subsequent mutations resulting in progression toward melanoma.[5]

Accordingly, *TERT*-p mutations in otherwise benign nevi should be interpreted with caution and are not synonymous with malignancy. *TERT*-p hotspot mutations have been identified in an acquired dermal and an acquired compound nevus, both in adults >50 years of age. In this context, *TERT*-p mutant subclones may be indicative of early UV-induced transformation.[6] Two examples of dysplastic nevi with moderate atypia based on consensus diagnosis by expert dermatopathologists also harbored *TERT*-p hotspot mutations.[7]

Focused primarily on molecular analysis, this review examines the roles of *TERT* and *TERT-p* in pathogenesis and their potential diagnostic and prognostic utility in cutaneous melanoma. The majority of the citied studies evaluated *TERT*-p mutational status by PCR amplification followed by direct Sanger sequencing.

2 | NONACRAL MELANOMA INCLUDING CHRONIC SUN DAMAGE AND NON-CHRONIC SUN DAMAGE MELANOMA

2.1 | Pathogenesis

In multiple series primarily composed of primary chronic sun damage (CSD) and non-CSD melanomas, *TERT*-p mutations (predominantly UV signature, including hotspot and tandem mutations) have been detected in 22% to 78% of tumors,[8-17] and Heidenreich et al found that their detection correlates with increased TERT mRNA expression.[8] *TERT*-p UV-signature mutations correlate with chronic[8,17] sun exposure, and both the −124:C > T and the −146 C > T hotspot mutations are associated with the CSD melanoma subtype (lentigo maligna, LM).[15] UV-signature mutations also result from intermittent[8,9] sun exposure and are associated with non-CSD melanomas, including superficial spreading melanoma (SSM)[9] and nodular melanoma (NM) subtypes.[8-10,15,16] Accordingly, *TERT*-p hotspot mutations are also significantly associated with Caucasian race, location on the upper extremities or head/neck, and nonacral tumors.[16,17] Less common non-UV-signature mutations, such as somatic −57 A > C mutations previously described as familial melanoma mutations, have also been detected in CSD and non-CSD melanomas by Heidenreich et al.[8] Concurrent *BRAF* mutations are significantly associated with *TERT*-p mutations in CSD and non-CSD melanomas,[10,11,14,15] particularly *BRAF* 600 including *BRAF* V600E mutations.[8,9] *TERT*-p UV-

TABLE 1 Nonacral melanoma including CSD and non-CSD melanoma[8-18]

Author and year	Number of cases	Demographic	Follow-up	Major findings
Heidenreich et al[8]	264	Adults		*TERT*-p mutations in 38% of melanomas associated with Breslow thickness >2 mm, vertical growth phase, ulceration, LNM and distant metastasis, *BRAF* 600 or *NRAS* mutations, and TERT mRNA expression
Populo et al[9]	116	Adults with mean age 60 y	54-57 mo (mean)	*TERT*-p UV-signature mutations in 22% associated with increased Breslow thickness, ulceration, mitotic index, *BRAF* V600E mutation, and decreased disease-free and overall survival; IHC (nuclear and cytoplasmic expression) did not correlate with prognostic features or survival
Macerola et al[10]	53	Adults with mean age 58 y (range 20-87)		*TERT*-p mutations in 38% of tumors; concomitant *TERT*-p and *BRAF* mutations in 21% associated with Breslow depth, mitotic index, ulceration, absence of regression, and LNM
Nagore et al[11]	300		47 mo (median)	*TERT*-p mutations in 38.7% of tumors; concomitant *TERT*-p and *BRAF* or *NRAS* mutations associated with shorter melanoma-specific survival; mutations did not negatively impact prognosis in carriers of rs2853669 polymorphism
Nagore et al[12]	285			*TERT*-p mutations with or without concomitant *BRAF* or *NRAS* mutations in 43% of tumors associated with fast-growing[a] tumors; mutations had less impact on growth rate in carriers of rs2853669 polymorphism
de Unamuno Bustos et al[16]	100			*TERT*-p mutations in 30% associated with absence of regression, increased Breslow depth, ulceration, and mitotic index
Roh et al[13]	30			*TERT*-p hotspot mutations in 27% but not prognostic; concomitant *TERT*-p and *BRAF* V600E mutations associated with decreased overall survival
Bai et al[14]	615		29 mo (median)	*TERT*-p mutations in CSD and non-CSD melanomas were not associated with prognostic features or survival
Andres-Lencina et al[15]	287			*TERT*-p hotspot or tandem mutations in 41% associated with Breslow depth >2 cm, mitotic index, concomitant *BRAF* or *NRAS* mutations, and disease recurrence; *TERT*-p −124:C > T hotspot and −138/−139 CC > TT tandem mutations associated with tumor stage > IB and disease-specific death; tandem mutation associated with worse disease-free and melanoma-specific survival
Osella-Abate et al[18]	105: 42 primary tumors; 46 locoregional metastases; 17 visceral metastases			*TERT*-p mutations in 70.4% associated with visceral metastasis as the first site of disease progression in non-CSD melanoma of the trunk
Thomas et al[17]	Primary melanomas; nevi; indeterminate proliferations			*TERT*-p hotspot mutations were 98.6% specific and 77.9% sensitive for the diagnosis of melanoma; hotspot mutations were not associated with prognostic pathologic features

Abbreviations: CSD, chronic sun damage; IHC, immunohistochemistry; LNM, lymph node metastasis; non-CSD, non-chronic sun damage; *TERT*-p, *TERT* promoter; UV, ultraviolet.

[a]≥0.5 mm Breslow thickness/mo.

signature mutations are also associated with concurrent *NRAS* mutations.[8,11,15] While *BRAF* and *NRAS* mutations are early events, *TERT*-p mutation and subsequently increased telomerase expression may facilitate stabilization of a transformed genome (Table 1).[8]

2.2 | Diagnosis

Thomas et al identified *TERT*-p hotspot and UV-signature tandem mutations in 78% of primary melanomas, 1.4% of melanocytic nevi, and 5% of diagnostically equivocal melanocytic proliferations. Over 80% of tumors in this series were CSD or non-CSD melanomas.[17] *TERT*-p hotspot and tandem mutations were 98.6% specific and 77.9% sensitive for the diagnosis of melanoma, with an overall accuracy of 87.3%. Of note, these figures do not account for *TERT*-p mutations that do not form ETS/TCF sites: 9.7% of nevi and 2.3% of melanomas harbored "non-ETS" mutations. *TERT*-p hotspot mutational status did not differ significantly between CSD (LM) and non-CSD (SSM, NM).[17] By contrast, in a Korean series of 12 CSD melanomas and 18 non-CSD melanomas, Roh et al observed *TERT*-p hotspot mutations in only 33.3% and 22.2% of tumors, and none of these tumors harbored the CC > TT tandem mutation. Differences in race, geography, and sample size may have contributed to these contrasting results.[13]

2.3 | Prognosis

Including hotspot mutations, *TERT*-p mutations have been associated with increased patient age at diagnosis,[15] particularly ≥65 years.[8,17] Increased Breslow thickness,[9,10,16] specifically >2 mm,[8,15] is also associated with hotspot mutations. Vertical growth phase and advanced tissue pathologic staging (tumor stage >IB) correlate with *TERT*-p mutational status.[8,10,15] Compared to WT tumors, ulceration,[8,9,15,16] and higher mitotic rates[9,10,15,16] (≥1 mm^2) are also more frequent in *TERT*-p mutants. Compared to *TERT*-p and *BRAF* wild-type tumors, concurrent mutations affect more prognostic variables than either mutation alone, including increased Breslow thickness, higher mean mitotic rate, and ulceration.[10] *TERT*-p mutations are significantly associated with the absence of regression,[10,16] particularly in the context of concomitant *TERT*-p and *BRAF* mutations.[10] Unamuno Bustos et al studied *TERT*-p mutations in 110 melanomas, the largest group showing no regression (47%), 41% with partial regression, and 12% with >50% or extensive regression. In contrast, all tumors with extensive regression were WT for *TERT*-p, suggesting that an absence of telomerase expression may permit telomere shortening and subsequent regression.[16] However, in a recent study of diagnostic utility with predominantly nonacral primary melanomas, there was no association between *TERT*-p hotspot mutations and Breslow thickness, ulceration, mitotic index, American Joint Committee on Cancer tumor stage, regression, or tumor infiltrating lymphocytes.[17]

Lymph node metastasis[8,10] and distant metastasis[8,18] were associated with *TERT*-p mutations in CSD and non-CSD melanomas. Among *TERT*-p mutants, the number of mutations correlated with the risk of metastasis,[8] and concurrent *TERT*-p and *BRAF* mutations affected the risk of lymph node metastasis more than either mutation alone.[10] *TERT*-p mutant tumors were significantly associated with primary tumor location on the trunk with subsequent visceral metastasis as the first site of progression in a study by Osella-Abate et al.[18]

TERT-p mutations have been significantly associated with decreased disease-free and overall survival, although not as an independently prognostic factor based on multivariate analysis.[9] Additionally, two more recent series did not identify a significant correlation between *TERT*-p mutational status and overall survival in CSD and non-CSD melanomas.[13,14] While the prognostic value of *TERT*-p mutational status alone is equivocal in this context, the presence of concomitant *TERT*-p and *BRAF*[11,13] or *NRAS*[11] mutations was significantly associated with decreased survival compared to WT tumors. In a study by Nagore et al, tumors harboring both *TERT*-p and *BRAF* or *NRAS* mutations were associated with fivefold decreased melanoma-specific survival compared to tumors without any of these mutations.[11] ETS transcription factors are downstream targets in the *BRAF* pathway, suggesting how coexistent mutations in *BRAF* and *TERT*-p may confer poorer prognosis or more aggressive behavior.[10]

In patients with CSD or non-CSD melanomas, the variant allele rs2853669 polymorphism (−245 T > C) may modify the prognostic impact of *TERT*-p mutations.[11,12] The variant allele of this polymorphism disrupts the ETS2 site, inhibits binding of c-Myc to E-box, and results in decreased promoter and telomerase activity.[11] This is particularly significant given that this variant allele frequency may be as high as 50%.[19] *TERT*-p mutations, with or without concurrent *BRAF/NRAS* mutations, did not reduce survival in patients who were carriers of the variant allele rs2853669 polymorphism.[11] Nagore et al separated a series of predominantly nonacral melanomas were separated into two groups: fast growing (≥0.5 mm/mo) vs slow growing (<0.05 mm/mo). Growth rate was calculated by dividing Breslow depth by the time elapsed between clinical suspicion and excision. *TERT*-p mutations, with or without either concurrent *BRAF* or *NRAS* mutations, were twice as common in fast growing compared to slow-growing melanomas. This effect on tumor growth was greater in patients who were noncarriers for the variant allele rs2853669 polymorphism.[12] Somatic *BRAF* mutations are also less frequent in carriers of the variant allele rs2853669 polymorphism and *TERT*-p WT or mutant melanomas.[19]

Less common *TERT*-p mutations may confer greater genomic instability than hotspot mutations. Andrés-Lencina et al compared the prognostic impact of the hotspot mutations and the −138/−139 CC > TT tandem mutation in a recent series. The tandem mutation was significantly associated with all of the prognostic features correlated with the −124:C > T hotspot mutation: Breslow depth >2 cm, mitotic index, disease recurrence, tumor stage >IB, and disease-specific death. Although the hotspot −124:C > T *TERT*-p mutation results in higher promoter activity, the −138/−139 CC > TT tandem mutation was associated with worse disease-free and melanoma-specific survival separately or in combination with *BRAF/NRAS* mutations.[15]

3 | ACRAL MELANOMA

3.1 | Pathogenesis

In primary acral melanomas, *TERT* copy number gains (≥2) have been detected in 24% to 44.9% of cases.[20-22] Amplifications (defined by gene copy number: chromosome control ratio ≥2) of *TERT* have been identified by fluorescence in situ hybridization (FISH) in up to 21% of acral melanomas. The majority of *TERT* amplifications are copy number transitions within 40 kb upstream of *TERT*.[23,24] *TERT*-p hotspot mutations are uncommon in primary cutaneous acral melanomas and have only been detected in 4.2% to 19% of tumors (Table 2).[8,13,23,25,26,27]

3.2 | Diagnosis

Diaz et al evaluated TERT copy number gains in acral melanomas and nevi. While infrequent in the invasive component of acral melanomas, copy number gains were identified within 100% of precursor MIS evaluated. This discordance is most likely attributable to *field cells*: clonally related melanocytes which arise in nonlesional epidermis before the appearance of precursor lesions such as MIS. Thus, the *TERT* copy numbers and amplifications observed in the invasive component are more likely to be representative of tumorigenesis in acral melanoma.[22] In contrast, no copy number gains were identified in 24 acral nevi. IHC is less specific in this context; nuclear reactivity was only detected up to 20% of cells in acral melanomas, failed to correlate with amplification, and was also detected in up to 10% of cells in acral nevi.[22]

3.3 | Prognosis

TERT amplifications were significantly associated with decreased overall survival in acral melanoma.[24] Additionally, *TERT* copy number gains in a series of 257 acral melanomas were associated with increased relapse-free survival following adjuvant therapy with high-dose interferon α-2b therapy; ulceration and tumor thickness >4 mm were associated with >4 copy gains.[21] In a large cohort of 1201 acral melanomas with median follow-up of 29 months, Bai et al found that *TERT*-p mutational status did not correlate with survival. Other series confirmed the lack of prognostic value for *TERT*-p mutational status in acral melanoma,[13,14,26,27] although acral melanomas harboring *TERT*-p

TABLE 2 Acral melanoma[8,13,14,20-27]

Author and year	Number of cases	Demographic	Follow-up	Major findings
Puig-Butille et al[20]	17	Adults (age range 34-86 y)		*TERT* copy number gains in 31%
Griewank et al[26]	42			*TERT*-p mutations in 19% but not an independent prognostic factor
Liau et al[25]	40:23 primary tumors; 17 metastases		30 mo (mean)	*TERT*-p UV-signature mutations in 6% of primary AMs but none of metastases
Heidenreich et al[8]	24			*TERT*-p mutations in 4.2%
Diaz et al[22]	58:34 AMs; 24 AN	AM: median 67 y (range 39-91) AN: median 37 y (range 16-64)		*TERT* copy number gains in 24% of AMs and 100% of precursor MIS, but absent in AN; Nuclear expression by IHC did not distinguish AMs and AN
Diaz et al[24]	43	Adults, median age 71 y (range 39-95)		*TERT* amplifications in 21% of AMs significantly associated with decreased overall survival
Vazquez et al[27]	43		35.5 mo (mean)	*TERT*-p hotspot mutations found in 7% of AMs but did not correlate with survival
Bai et al[14]	1201		29 mo (median)	*TERT*-p hotspot mutations in 11.4% of AMs but did not correlate with survival
Roh et al[13]	46			*TERT*-p hotspot mutations found in 10.9% of AMs and associated with increased Breslow thickness but not an independent prognostic factor
Yu et al[21]	573			*TERT* copy gains in 44.9% of tumors; associated with ulceration, Breslow thickness >4 mm, and decreased relapse-free, but not overall, survival
Yeh et al 2019[23]	197			*TERT*-p mutations in 5.3% and *TERT* amplifications in 10.7% of tumors

Abbreviations: AM, acral melanoma; AN, acral nevi; IHC, immunohistochemistry; MIS, melanoma in situ; *TERT*-p, *TERT* promoter; UV, ultraviolet.

hotspot mutations may have greater Breslow thickness compared to WT tumors.[13] Owing to the predominance of the acral melanoma subtype in Asian patients, *TERT*-p mutational status appears less prognostically relevant in this population.[14]

4 | METASTATIC MELANOMA AND MELANOMAS OF UNKNOWN PRIMARY

4.1 | Pathogenesis and diagnosis

TERT-p mutations have been identified in over half of primary and metastatic melanomas, without significant difference in mutational frequency between primary and metastatic tumors.[28,29] *TERT*-p mutations are not essential for tumor progression after locoregional metastasis,[29] and driver mutations in *CDKN2A*, *PTEN*, or *TP53* are more likely relevant than *TERT*-p at this stage.[30] Within matched primary and metastatic tumors, discordant *TERT*-p mutational status has been observed in 24% to 32% of cases.[29,30] However, the discordance rate decreases significantly after accounting for tumor heterogeneity and subsequent metastasis. Among discordant pairs, mutational loss (62%) is more frequent than acquisition (38%) in the metastatic tumor.[30] IHC does not appear valuable in this context: cytoplasmic TERT expression did not correlate with *TERT*-p mutational status and was discordant in 42% of matched pairs.[29] In contrast to primary mucosal melanomas, Egberts et al identified frequent TERT-p hotspot mutations in melanomas of unknown primary, supporting a cutaneous origin (Table 3).[31]

4.2 | Prognosis

Given the high prevalence of UV-signature mutations in metastatic melanomas, *TERT*-p mutations may confer greater metastatic potential. In 203 nonacral cutaneous metastatic melanomas from 170 distinct patients with a median follow-up time of 4 years, Ekedahl et al found that 81% of patients had tumors with *TERT*-p mutations.[32] In four series, *TERT*-p mutations in metastatic tumors were not associated with reduced survival.[28,29,30,32] While adverse tumor characteristics—thicker Breslow depth and higher mitotic rate by PHH3—were correlated with *TERT*-p mutational status in one study, most series have not reproduced this association.[28,29,30,32] Thus, *TERT*-p WT metastases may produce increased telomerase expression without *TERT*-p mutations or depend on other driver mutations for progression.[28,30,32] Similarly, there was no association between *TERT*-p mutations and survival in patients with melanoma of unknown primary.[31]

5 | MULTIPLE PRIMARY MELANOMAS

Pellegrini et al evaluated the mutational status of *TERT*-p in 97 melanomas from 44 patients with multiple primary melanomas. Almost all

TABLE 3 Metastatic melanoma, MUP, and MPM[28-33]

Author and year	Number of cases	Demographic	Follow-up	Major findings
Egberts et al[31]	MUPs			*TERT*-p hotspot mutations found in 67% of MUPs but did not demonstrate prognostic value
Ekedahl et al[32]	203 nonacral cutaneous metastatic melanomas (170 distinct patients)		4 y (median)	*TERT*-p UV-signature mutations in metastases from 81% of patients but not a prognostic factor
Ofner et al[28]	115 primary and metastatic melanomas			*TERT*-p mutations identified in 54.8%; no difference in frequency between primary and metastatic tumors; no prognostic value
Hughdahl et al[29]	266: 194 primary nodular melanomas; 72 matched locoregional metastases			*TERT*-p mutations identified in 68% of nodular melanomas and 64% of locoregional metastases; mutational status associated with Breslow depth and mitotic index but not survival; mutational status discordant in up to 24% of matched cases; IHC (cytoplasmic expression) did not correlate with mutational status but did correlate with Breslow depth and reduced survival; IHC discordant in 42% of matched cases
Yang et al[30]	43 pairs (matched primary and metastatic melanomas)			*TERT*-p mutations identified in 43% of primary melanomas and 29% of metastases but not a prognostic factor; discordant mutational status in 32%, most often due to loss of mutation
Pellegrini et al[33]	97 non-CSD melanomas (44 patients with MPM)			*TERT*-p mutations in 19.6% of tumors; intrapatient mutational discordance was 45%

Abbreviations: IHC, immunohistochemistry; MPM, multiple primary melanomas; MUP, melanoma of unknown primary; non-CSD: non-chronic sun damage; *TERT*-p: *TERT* promoter.

TABLE 4 Atypical spitzoid neoplasms: AST and SM[34-38]

Author and year	Number of cases	Demographic	Follow-up	Major findings
Lee et al[36]	56	Pediatric and adult; mean age 14.6 y (range 2-61)	Mean 32.5 mo	*TERT*-p hotspot mutations in 7% associated with distant metastasis and fatal outcome
Lu et al[37]	5	Pediatric	Mean 32 mo	Single SM with *TERT*-p hotspot mutation associated with distant metastasis and fatal outcome
Wu et al[38]	7	Pediatric; age range 2-14 y	Median 20 mo	Two tumors with *TERT*-p hotspot mutations associated with distant metastasis and fatal outcome
Lee et al[34]	9	Pediatric and adult		*TERT*-p hotspot mutations and increased TERT mRNA expression associated with SM
Fan et al[35]	9	Pediatric and adult		*TERT*-p CpG methylation did not distinguish SM and AST

Abbreviations: AST, atypical spitz tumor; SM, spitzoid melanoma; *TERT*-p, TERT promoter.

the tumors assayed were non-CSD melanoma, and 75% were metachronous. *TERT*-p hotspot mutations were found in 19.6% of tumors, with a significant decrease in mutational frequency between first and subsequent melanomas. The rate of intrapatient *TERT*-p mutational discordance was 45%, highlighting the somatic heterogeneity of multiple primary melanomas (Table 3).[33]

6 | ATYPICAL SPITZOID NEOPLASMS

6.1 | Diagnosis and pathogenesis

TERT-p sequencing may aid in the distinction of atypical Spitz tumor (AST) from spitzoid melanoma. In a series of three spitzoid melanomas and six ASTs from pediatric and adult patients, Lee et al demonstrated hotspot *TERT*-p mutations in all three spitzoid melanomas but in none of the borderline spitzoid neoplasms. In a metastasis secondary to spitzoid melanoma with fatal outcome, RT-PCR demonstrated significantly elevated *TERT* mRNA expression, while *TERT* mRNA was undetectable or expressed at low levels in nine ASTs.[34] Epigenetic upregulation by DNA methylation is unlikely to helpful in this context, as none of these atypical spitzoid neoplasms demonstrated *TERT*-p CpG methylation (Table 4).[35]

6.2 | Prognosis

TERT-p mutational status may also support risk stratification among ASTs and spitzoid melanomas, given that the frequent presence of lymph node metastasis in this context does not predict extranodal disease or death. In a study of 56 adult and pediatric patients with ASTs or spitzoid melanomas by Lee et al, tumors harboring *TERT*-p hotspot mutations were associated with distant metastasis and fatal outcome. Ninety-three percent of patients had tumors with WT *TERT*-p and were alive without distant metastasis at a follow-up period of 32.5 months.[36] These findings were reproduced in two subsequent smaller series of pediatric patients, in whom tumors with WT *TERT*-p did not progress beyond lymph node metastasis.[37,38] In atypical spitzoid neoplasms, the presence of a *TERT*-p hotspot mutation has also been associated with age ≥10, mitotic rate > 5 mm^2, and ulceration.[36]

7 | CONVENTIONAL AND NEVOID MELANOMAS IN PEDIATRIC PATIENTS

7.1 | Pathogenesis

Similar UV-signature *TERT*-p mutations drive tumorigenesis in pediatric and adult conventional melanoma. In a series of 15 conventional melanomas from pediatric patients, Lu et al identified *TERT*-p mutations in 92% of sequenced tumors, and 80% of these mutations were UV signature. The single conventional melanoma with wild-type *TERT*-p was an AM. Additionally, 87% of tumors contained an activating *BRAF* V600 mutation.[37] *TERT*-p mutant status in pediatric conventional melanoma correlates with—but is not required for—telomerase expression: *TERT*-p hypermethylation may also increase *TERT* mRNA expression.[34,35] In a series of 19 conventional melanomas in pediatric patients, *TERT*-p hotspot mutations and hypermethylated CpG sites were identified in 53% and 42% of tumors, respectively. Rearrangements involving the *TERT* locus were also demonstrated by FISH in two of eight conventional melanomas. In contrast, none of these aberrations that increase telomerase expression were present in two nevoid melanomas (Table 5).[39]

7.2 | Prognosis

TERT-p methylation—alone or with concomitant *TERT*-p hotspot mutation—is associated with reduced recurrence-free survival, but not overall survival. Of note, *TERT*-p mutations alone did not predict adverse outcomes in pediatric conventional melanoma in the largest series to date by Seynnaeve et al.[39]

TABLE 5 Conventional and nevoid melanomas in pediatric patients[34,35,37,39]

Author and year	Number of cases	Demographic	Follow-up	Major findings
Lu et al[37]	15	Median age 16 y (range 11-20)		Twelve of thirteen tumors with *TERT*-p mutations, predominantly UV-signature
Lee et al[34]				*TERT*-p mutations or *TERT*-p hypermethylation can increase TERT mRNA expression in pediatric CM
Fan et al[35]				*TERT*-p mutations or *TERT*-p hypermethylation can increase TERT mRNA expression in pediatric CM
Seynnaeve et al[39]	21 (19 CM, 2 nevoid melanoma)	Median age 21 y (range 13-25)		*TERT*-p mutations, hypermethylation, and increased TERT mRNA expression in CMs; hypermethylation associated with reduced recurrence-free survival

Abbreviations: CM, conventional melanoma; *TERT*-p, TERT promoter; UV, ultraviolet.

TABLE 6 Melanoma arising in GCN[34,35]

Author and year	Number of cases	Demographic	Follow-up	Major findings
Fan et al[35]	Six: (three melanomas; three proliferative nodules)			Hypermethylation of WT *TERT*-p and increased TERT mRNA expression observed in melanoma but absent in proliferative nodules
Lee et al[34]	Two metastatic melanomas			Metastases from melanoma arising in GCN also demonstrate increased TERT mRNA expression

Abbreviations: GCN, giant congenital nevi; *TERT*-p, *TERT* promoter; WT, wild type.

8 | MELANOMA ARISING IN CONGENITAL NEVI

Methylation-dependent epigenetic upregulation of *TERT* may play a role in the pathogenesis of melanoma arising in giant congenital nevi (GCN) and allow differentiation from proliferative nodules in GCN. In three melanomas with WT *TERT-p* arising in congenital nevi, next-generation sequencing of *TERT*-p demonstrated that almost all CpG sites sequenced were highly methylated. In contrast, the same region of *TERT*-p was predominantly unmethylated in three GCN with proliferative nodules. *TERT*-p methylation also correlated strongly with telomerase expression assayed by *TERT* mRNA in situ hybridization (ISH).[35] In a follow-up study, real-time (RT) quantitative PCR of metastases secondary to two of these melanomas arising in GCN demonstrated a 20- to 27-fold increase in *TERT* mRNA expression (Table 6).[34]

9 | DESMOPLASTIC MELANOMA

In a series of 76 desmoplastic melanomas, Yang et al identified *TERT*-p mutations in 34% of tumors. Desmoplastic melanomas characterized by at least 90% paucicellular spindle cells and stromal fibrosis were classified as pure, while those with greater than 10% cellular foci or epithelioid cytomorphology and without stromal fibrosis were classified as mixed.[40,41,42] Mixed desmoplastic melanoma was three times more likely than pure desmoplastic melanoma to harbor *TERT*-p hotspot mutations as well as other mutations upstream of the ATG start site. Thus, loss of telomere integrity may be more relevant to the pathogenesis of mixed desmoplastic melanoma than of pure desmoplastic melanoma. *TERT*-p mutational status is not significantly associated with ulceration, the presence of mitotic figures, perineural invasion, *BRAF* mutations, or Breslow depth in mixed or pure desmoplastic melanoma.[43]

10 | DIFFERENTIATION OF RECURRENT MELANOMA FROM RECURRENT NEVI

In a study by Walton et al, *TERT*-p hotspot mutations were identified in four of six recurrent melanomas and none of 17 recurrent nevi, suggesting the diagnostic specificity of *TERT*-p sequencing in challenging cases with overlapping histopathological features such as dyscohesion of the dermoepidermal junction, epidermal effacement, and nuclear atypia.[7]

11 | CONCLUSION

The exact role of molecular analysis of *TERT* and *TERT*-p analysis in routine practice still needs to be refined. There is data demonstrating that *TERT*-p mutations are relatively sensitive and specific in differentiating some subsets of melanoma from nevi. However, the greatest utility for *TERT*-p mutational analysis is for borderline lesions that are not obviously benign or malignant by histopathological examination alone. In this area, there is still a lack of robust studies that correlate

TERT-p mutational status with outcome data in histopathologically ambiguous melanocytic lesions for which this molecular study may be helpful.

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CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

ORCID

Kiran Motaparthi https://orcid.org/0000-0003-0562-0826
Tricia A. Missall https://orcid.org/0000-0001-9062-0606
Claudia I. Vidal https://orcid.org/0000-0003-2672-4974
Maxwell A. Fung https://orcid.org/0000-0003-1771-8257
Patrick O. Emanuel https://orcid.org/0000-0003-1068-6969

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