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Pathology and Genomics of Pediatric Melanoma: A Critical Re-examination and New Insights

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Abstract

The clinicopathologic features of pediatric melanoma are distinct from those of the adult counterpart. For example, most childhood melanomas exhibit a uniquely favorable biologic behavior, save for those arising in large/giant congenital nevi. Recent studies suggest that the characteristically favorable biologic behavior of childhood melanoma may be related to extreme telomere shortening and dysfunction in the cancer cells. Herein, we review the genomic profiles that have been defined for the different subtypes of pediatric melanoma and particularly emphasize the potential prognostic value of *TERT* alterations for these tumors.

Keywords

Pediatric melanoma; spitzoid melanoma; childhood melanoma; melanoma arising in association with large/giant congenital nevi

INTRODUCTION

The pathologic diagnosis of pediatric melanoma is contingent upon integrating certain clinical information, is increasingly reliant on ancillary and molecular diagnostic testing, and often can only be made after consultation with individuals or institutions experienced in this difficult diagnosis.[1–4] Pediatric melanoma differs in unique ways from adult melanoma and warrants special recognition.[5,6] Further, even between prepubescent and postpubescent melanoma there are key differences with respect to those subtypes most commonly observed and their biological behaviors.[7–12] Because of these reasons, patient age is used to classify pediatric melanoma into i) congenital, ii) childhood (prepubescent; arbitrarily aged < 10 years), and iii) adolescent (postpubescent; arbitrarily aged 11 to 19 years) subtypes.[13]

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Genome-wide sequencing studies have defined the genomic profiles of these subtypes of pediatric melanoma.[14] These studies, combined with epigenetic analyses, point to dysregulation of the *telomerase reverse transcriptase (TERT)* gene as an intriguing explanation for the varied clinical behavior observed between childhood and adult melanoma.[15–17] The purpose of this review is to provide an update on the current knowledge in the molecular biology of pediatric melanoma. It summarizes critically important clinicopathologic factors in the diagnosis of pediatric melanoma, discusses the genomics of pediatric melanoma with an emphasis on the potential prognostic value of telomerase reactivation in pediatric melanoma, and provides an integrated overview of clinical, pathologic, and genomic aspects of specific pediatric melanoma subtypes.

CLINICOPATHOLOGIC CONSIDERATIONS

Certain clinical data and pathologic considerations are essential in evaluating pediatric melanocytic lesions. These include: age of the patient, anatomic site of the lesion, knowledge about preexisting conditions, such as presence of congenital or numerous acquired melanocytic nevi or genetic disorders, detailed information about the clinical nature and evolution of the lesion, and acute awareness of potential histologic mimics of melanoma.

Patient Age

Prepubertal and postpubertal pediatric melanoma are significantly different, with the latter sharing many biological properties with adult melanomas.[7–10] In prepubescent children, most cases of melanoma encountered are pediatric spitzoid melanomas/atypical Spitz tumors. These are considered low-grade/borderline malignant neoplasms that rarely result in clinically apparent distant metastases.[7,18,19] A second, biologically distinct form of melanoma in prepubescent children that arises in association with large/giant congenital nevi, or *de novo*, is highly aggressive and rapidly lethal.[14,20,21]

In postpubescent pediatric patients (adolescents), two major subtypes of melanomas are most commonly encountered. The first subtype is conventional adult melanoma, which shares similar morphologic (superficial spreading and nodular) and molecular features with adult melanoma (details in the next section).[14,17] The second subtype is spitzoid melanoma, which exhibits a heterogeneous clinical behavior and an unpredictable clinical course in adolescents.[22]

Histologic Mimics

The following histologic mimics may occur with some frequency in the pediatric population and are prone to be diagnosed erroneously as pediatric melanoma: (1) Spitz nevi and atypical variants[1], (2) proliferative nodules in congenital nevi,[23,24] (3) acquired melanocytic nevi from particular anatomic sites, such as the scalp,[25,26] genital area, [27,28] conjunctiva,[29] and acral surfaces;[30] and (4) melanocytic nevi with pagetoid melanocytosis, Reed nevus, deep penetrating nevus, epithelioid blue nevus/pigmented epithelioid melanocytoma and cellular blue nevus.[2,31]

Predisposing Conditions

Pre-existing conditions, such as congenital nevi,[32,33] familial melanoma,[34,35] an atypical nevus phenotype,[34] neurocutaneous melanosis,[36,37] xeroderma pigmentosum (DNA repair defects),[38,39] or immunosuppression [13,40,41] correlate with an increased risk for pediatric melanoma.

General Histopathologic Considerations

The histopathologic criteria used for diagnosis of pediatric melanoma are essentially the same as those used for adult cases. Melanomas can be distinguished from nevi by some combination of the following characteristics: large size (lesion diameter >6 mm and especially >1 cm), asymmetry, poorly demarcated lateral borders, ulceration, lack of maturation, confluence and high density of melanocytes in “sheet-like” arrangements, high dermal mitotic rate (>6 mitoses/mm²), mitoses in the deepest portion of the dermal component, dusty cytoplasmic melanin, and severe cytologic atypia.[1,2]

PEDIATRIC MELANOMA TUMORIGENESIS

Despite the inherently complex genomic profile of malignant melanocytes, alterations in a defined set of molecular pathways are thought to drive melanoma tumorigenesis.[42,43] These critical pathways are dysregulated stepwise, often in the following sequence for adults [44]: (1) activation of the RAS/RAF/mitogen-activated protein (MAP) kinase signaling pathway by an oncogenic driver, (2) telomerase reactivation, (3) inhibition of the p16-retinoblastoma (p16/RB) pathway, and (4) alterations of the PTEN and the phosphatidylinositol 3-kinase (PTEN-PI3K) pathway. However, the same order of events might not occur in childhood melanomas, which are biologically distinct.[15,17]

Melanoma Oncogenes and Tumor Suppressor Genes

Adult melanomas are divided into 4 mutually exclusive molecular subgroups based upon the oncogenic driver that initiates tumorigenesis: (1) activating mutations in *BRAF*, (2) activating mutations in the *RAS* family (most commonly *NRAS*), (3) inactivating mutations in *NFI* (negative regulator of the RAS pathway), and (4) the “triple wild-type” category, encompassing melanomas lacking *BRAF*, *NFI*/*KRAS*, or *NFI* mutations.[45,46] Such oncogenic aberrations result in constitutive activation of the MAP kinase pathway.[46,47] The PI3K-AKT pathway is also commonly dysregulated in melanomas. In *BRAF*-mutant melanomas, this is accomplished through alterations of *PTEN* (negative regulator of PI3K-AKT),[48–50] while in *NRAS*-mutant melanomas this pathway is directly activated through *NRAS*, which activates both the MAP kinase and the PI3K-AKT pathways.[51]

In pediatric melanoma, *BRAF* is the predominant driver of conventional adult-subtype melanoma whereas *NRAS* is almost unique to melanomas arising in association with large/giant congenital melanocytic nevi (CMN).[14,17] In contrast, kinase fusions are observed in 39% of spitzoid melanomas.[15,52,53]

Without additional alterations, tumor progression is restricted by replicative or oncogene-induced senescence.[43,54] For example, induction of p16^{INK4a} (CDKN2A) in nevi inhibits

cell cycle progression via a negative interaction with cyclin-dependent kinase 4 (CDK4) and CDK6, thereby activating the RB family of tumor suppressors to induce senescence.[54] Melanoma cells may escape senescence by abrogation of the p16-RB pathway,[43] often through loss, inactivating mutation or epigenetic alteration of *CDKN2A* (~60%) or, less commonly, through mutation of *CDK4* or other related genes.[55]

Telomerase Reactivation in Pediatric Melanoma

Somatic cells have limited replicative capacity because telomere length is progressively shortened through iterative cycles of replication,[56] which activates cell cycle checkpoints resulting in replicative senescence. Acquiring a means to maintain telomere length is therefore an essential step in the development of cancer cells, known for their capacity for uncontrolled proliferation.[57] In 85%–90% of cancer cell lines and tissues, telomere shortening is countered by aberrant expression of the *TERT* oncogene. *TERT*, which is normally silenced in somatic cells, encodes the catalytic subunit of the telomere-lengthening enzyme telomerase.[58,59]

TERT is aberrantly expressed in cancer by several possible mechanisms. Promoter mutations activating *TERT* transcription are seen in approximately 70% of melanomas in adults.[44,60,61] These mutations occur mainly at two mutually exclusive positions that are 124 and 146 base pairs upstream of the start codon.[60,61] In melanoma, *TERT* expression can also be deregulated through aberrant promoter methylation,[16,17] or by structural rearrangement of the gene; the latter occur in a manner mutually exclusive of promoter mutation.[17,62] Such lesions in *TERT* occur commonly in malignant adult melanomas[60–62] and biologically aggressive adolescent melanomas,[17] but are not observed in low-grade pediatric melanomas.[15–17] Thus, a combination of *TERT* promoter mutation, hypermethylation, or rearrangement, in conjunction with other clinicopathologic parameters, holds promise as a prognostic marker for pediatric melanomas.

TERT promoter mutations are acquired before alteration of *CDKN2A* in adult invasive melanoma[44] however, this is not consistently seen in pediatric melanoma.[15,17] Biallelic loss of *CDKN2A* characterizes a subset of low-risk/borderline-risk spitzoid or nevroid melanomas which have not yet activated telomerase.[15,17]. It is possible that alterations in cell cycle checkpoint genes have allowed melanocytes to escape from replicative senescence and divide beyond the number of cell divisions typically allotted. Eventually, however, telomere shortening would induce telomeric crisis and cell death. This may explain the favorable outcome in otherwise morphologically malignant pediatric tumors which do not express *TERT* and are marked by chromosomal instability. These tumors are unable to maintain sufficiently long telomeres and therefore cannot sustain continued proliferation.

SUBTYPES OF PEDIATRIC MELANOMA

Conventional Adult Subtype Melanoma

Approximately 40%–50% of pediatric melanomas are histologically similar to adult melanomas.[2,4] For reasons that are unknown, this conventional adult subtype of melanoma rarely occurs in prepubescent children. In our experience, adult melanoma type can rarely

occur in peri-adolescence, in patients as young as 10 to 11 years of age, even in the absence of pre-existing conditions (Figure 1). These patients are typically Caucasians with fair skin, poor tanning ability, and numerous nevi.[63,64] More than two-thirds of pediatric patients with adult conventional subtype melanoma harbor variants of *Melanocortin 1 receptor* (*MC1R*), which is associated with fair, sun-sensitive skin and an increased susceptibility to melanoma.[65] Germline mutations in the melanoma susceptibility gene *CDKN2A*, which is responsible for 25%–50% of familial melanomas, are seen only in a small subset of sporadic early onset melanoma cases (1.6%).[14,35,41,66]

As seen in adult “superficial spreading” melanomas, these melanomas often develop via a radial or horizontal growth phase, which is initially intra-epidermal (*in situ*) or microinvasive melanoma. Metastatic disease is unlikely to occur until the tumor enters the vertical growth phase. Epithelioid melanoma cells are characteristically disposed in a pagetoid array and, given time, usually invade the papillary dermis. Although adult melanomas can be diagnosed by prominent pagetoid melanocytosis, a subset of nevi in children may display this feature thus diminishing its clinical utility. “Nodular” melanomas are defined by an almost exclusively bulky or cohesive invasive component and little or no associated lateral intraepidermal component (Figure 1). Melanomas of acral (glabrous) skin are rare in childhood.[2,4,67] Lentiginous melanocytic proliferation and upward migration of melanocytes are common in childhood nevi, particularly in glabrous skin. These changes must not be over-interpreted unless architectural disorder is prominent and cytological abnormalities occur throughout the breadth of the lesion. In one European study, only three of 145 Caucasian children with melanomas had involvement of the soles.[4] In general, lentigo malignant melanomas, typically seen in chronically sun damaged skin, do not occur in childhood except in xeroderma pigmentosum patients.[1,4]

Pediatric conventional adult subtype melanomas are genetically also very similar to adult melanomas.[14] Whole-genome and -exome sequencing of 15 pediatric conventional adult subtype melanomas showed that they were similar to adult tumors by (1) having an ultraviolet light-induced mutational burden with an abundance of C>T transitions at dipyrimidine sites, (2) often harboring activating mutations in the *BRAF* and the *TERT* promoter, and (3) frequently harboring inactivating mutations in *CDKN2A* and *PTEN* tumor suppressor genes upon *BRAF* activation.[14,68] In a follow-up study of adolescent melanomas, other mechanisms of *TERT* dysregulation (i.e., promoter hypermethylation and structural rearrangement) were found in a subset of tumors.[17]

Activated *BRAF* drives 85% or more of pediatric conventional melanomas and the presence of this signature should raise a strong suspicion of this subtype (Figure 1),[14,17] although we have observed *BRAF* mutations in some low-risk nevoid melanomas and rarely in pediatric spitzoid melanomas.[15,17] As the natural history of disease for pediatric conventional and adult melanomas is similar,[21,39,69–71] their management should likely be the same. The classification and stage grouping criteria, based on tumor thickness, mitotic rate, ulceration, and nodal metastases, according to the American Joint Committee on Cancer staging system used for adult cutaneous melanoma, are also applied to the pediatric counterpart.[72]

Spitzoid Melanoma

Spitzoid tumors including spitzoid melanoma are a distinct histologic variant of melanocytic neoplasms that are more commonly seen in individuals younger than 20 years. Despite a high incidence (~50%) of sentinel lymph node metastases in spitzoid neoplasms, these patients often have excellent outcomes and rarely develop distant disease.[15,22,73]

Reliable classification of spitzoid lesions such as Spitz nevus, atypical Spitz tumor, or spitzoid melanoma remains a challenge, especially in the pediatric population.[1,2,74,75] The principal question of what constitutes a “spitzoid” melanoma[75,76] can be addressed by clinical, histopathologic, and genetic criteria. Clinically, pediatric spitzoid melanoma is commonly amelanotic and presents as a pink, red papule, nodule, or polyp that clinically resembles a benign non-melanocytic lesion, such as pyogenic granuloma or wart. [6,10,77,78] Thus, the conventional ABCDE (asymmetry, border irregularity, color variation, diameter >6 mm, and evolution) criteria recommended as a visual guide for the detection of melanoma in adults may not reliably capture this melanoma subtype.[78]

Histopathologic features of spitzoid melanoma are first of all cytologic – the presence of prototypic enlarged epithelioid and/or spindle cells – but also architectural, such as the silhouette of the lesion often integrating a well-defined, dome-shaped configuration with epidermal hyperplasia, vertical disposition of melanocytes, and a wedge-shaped dermal architecture (Figure 2)[1,2,74–76,79].

An often neglected yet critical point is that not all melanocytic neoplasms with spindle/epithelioid cell features are spitzoid. Increasingly, genetic criteria can aid in the classification of spitzoid melanomas. For example, specific kinase gene fusions often occur in such melanomas but not in other spindle and epithelioid cell melanocytic neoplasms or conventional melanomas. [52,53] These events are found in approximately 50% of all spitzoid tumors (from nevus to melanoma), and involve the receptor tyrosine kinases *NTRK1*, *NTRK3*, *ALK*, *ROS1*, *RET*, or *MET*, or the serine-threonine kinase *BRAF* (Figure 2).[52,53,80,81] As these kinase fusions are present across the entire spitzoid spectrum, they are unlikely to determine the biology of these tumors.[15,52] Activating mutations in *HRAS* or loss/inactivation of *BAP1*, which occurs in a subset of Spitz nevus and atypical Spitz tumors, is mutually exclusive from kinase fusions.[82] Activating *BRAF* mutations are uncommon in spitzoid tumors, but occur in a subset of epithelioid spitzoid melanocytic tumors with *BAP1* loss.[83]

Another critical question is how spitzoid melanoma can be distinguished from atypical Spitz tumors. Essential histopathologic criteria for melanoma have been elaborated in an earlier section. In challenging cases, genetic criteria can aid in the evaluation of these neoplasms. A normal chromosomal copy number status or some changes limited to an isolated alteration (e.g., extra copies of 11p or gains involving 7q) point to the diagnosis of Spitz nevus or atypical Spitz tumor, respectively. On the other hand, multiple copy number changes as seen in conventional melanoma suggest the diagnosis of spitzoid melanoma.[84,85] These changes can be detected by fluorescence *in situ* hybridization (FISH) or comparative genomic hybridization (CGH). Even if the diagnosis is spitzoid melanoma, the majority of childhood tumors, at least in short-term follow-up, never develop clinically detectable

metastasis beyond local lymph nodes. In contrast, rare spitzoid neoplasms may result in distant metastases and death.[15,22,86] Thus, the crucial question of how the biological potential of a particular spitzoid lesion can be predicted remains unresolved.

Recent, preliminary studies suggest that the presence of *TERT* alterations may be useful in predicting clinical behavior.[15] In a series of 56 pediatric and adult patients (median age, 9 years) with atypical Spitz tumors or spitzoid melanomas, *TERT* promoter mutations were only detected in tumors from patients who developed visceral metastasis (two adolescents, ages 11 and 14; two adults).[15] The presence of these mutations correlated significantly with high risk parameters described by Barnhill *et al* for risk stratification of spitzoid lesions: Age ≥ 10 years, mitotic rate (i.e., >5 per mm²) and ulceration.[15,87].

One must be wary that the presence of promoter mutation alone cannot be used to predict elevated TERT mRNA levels, since other mechanisms of TERT upregulation are also possible in cancer cells. TERT mRNA quantification in biopsy samples has great prognostic potential but unfortunately is of limited practical application because such samples are often contaminated with stromal or non-tumoral tissue or have degraded RNA in old archival material. Thus, detecting any one of the three DNA-level *TERT* alterations mentioned earlier may be useful as a substitute for detection of elevated TERT mRNA levels.

The prognostic parameters used for staging in adult melanomas, such as Breslow thickness and nodal metastases, do not carry the same weight in predicting outcome in pediatric spitzoid melanoma/atypical Spitz tumor as in conventional melanoma.[7,19,72,73] In fact, because of their usually excellent prognosis, the question remains whether childhood spitzoid melanomas merit the designation of melanoma. It is true that the presence of a malignant process in these tumors is supported by the genomic instability suggested by frequent observations of multiple copy number changes that are detected by CGH or FISH, and the frequent biallelic loss/inactivation of *CDKN2A*, involved in replicative and oncogene-induced senescence. However, in the absence of *TERT* dysregulation, continuous cellular propagation in these cases eventually leads to extreme telomere shortening and eventually telomeric crisis,[88,89] which is characterized by end-to-end chromosomal fusions, chromosome breakage, and anaphase bridges. These cellular events may explain the changes in gene copy number and the morphologic features of malignancy commonly observed in such neoplasms.[88,89] Although replicative lifespan is limited in these non-immortal malignant cells, there still remains the potential to acquire the capacity to maintain telomere lengths, either through TERT expression or through a telomerase-independent Alternative Lengthening of Telomeres mechanism. It is well-known that clinically localized melanomas can recur with distant metastasis several years or even decades after removal of the primary tumor.[90] Although the incidence of such further progression is likely confined to a rare subset of cases, given the typically excellent outcomes of patients with pediatric atypical Spitz tumors/spitzoid melanomas, anecdotal reports of late recurrence[91] suggest that long-term follow-up is required to appreciate the full potential of this neoplastic process.

Melanoma Arising in Large/Giant Congenital Melanocytic Nevi

CMN are defined as pigmented lesions being present at birth. Large (predicted adulthood diameter, >20 – 40 cm) or giant (predicted adulthood diameter >40 cm or covering $\geq 5\%$ of

the skin surface) CMN carry an approximately 6% lifetime risk of malignant transformation.[32,33,92,93] More than half of the melanoma cases that are associated with these lesions occur before the age of 10 years.[2,32,33,92] Melanomas arising in large or giant CMN represent a highly aggressive subtype that is often associated with rapid progression and a fatal outcome.[14,20,94]

These are commonly of the small-cell type of melanoma, which can lead them to be misconstrued as a benign lesion. They are generally dermal and composed of epithelioid cells, spindle cells, or small round cells resembling a malignant small round “blue” cell tumor (Figure 3).[1,2,4] Cells can take on the appearance of lymphoblastic lymphoma or comparable “blastic” tumors.[1,2] Mitotic figures and nuclear debris are also commonly observed.

At the molecular level, these melanomas often harbor an activating mutation in *NRAS* at codon 61, acquired at the precursor lesion stage.[14,20,95] Alterations in *PTEN* are uncommon and *TERT* promoter mutations are not observed, at least in children.[14,16] Instead, *TERT* upregulation may be mediated through aberrant promoter methylation.[16,96]

It is important to realize that most nodular proliferations developing in large or giant CMN, particularly in younger children, are in fact benign.[1,2,4] However, it may be extremely difficult to histopathologically distinguish melanoma from benign proliferative nodules developing in CMN (Figure 3). Ulcerated proliferative nodules in neonates, often on the scalp, composed of small cells with a high mitotic activity, can be especially challenging to distinguish.[97] In rare instances, large/giant CMN are associated with contiguous involvement of lymph nodes that must be distinguished from nodal metastasis. These lesions can be distinguished from melanoma by CGH and *TERT* promoter methylation analysis. CGH analysis has revealed gains and losses of whole chromosomes in proliferative nodules, in contrast to the regional copy number variation that is observed in melanoma.[97,98] Unlike melanomas arising in CMN, which express high levels of *TERT* mRNA, proliferative nodules do not express *TERT* mRNA at detectable levels (Figure 3).[16] These melanomas are characterized by aberrant *TERT* promoter methylation, which can be detected by high-throughput bisulfite methylation sequencing or combined bisulfite restriction enzyme analysis.[96] Such epigenetic alterations are absent in proliferative nodules.

Congenital melanoma

Melanoma at birth or in early life is rare. There are three types of congenital melanoma.[1,2] The first type is maternal melanoma metastatic to the fetus.[99] In this case, neonates usually have widespread visceral metastases at birth and die within days to months. The placenta usually shows gross and histopathological evidence of metastatic melanoma. Because of the theoretical risk of transmission from mother to fetus, placentas should be examined for gross and histopathological evidence of metastatic disease with maternal history of invasive melanoma. When present, placental metastases rarely lead to congenital melanoma; some placental deposits also appear benign and may represent developmental arrests or “benign seeding” of the placenta.[100] Nonetheless, the finding of placental involvement by melanocytic deposits warrants careful examination and follow-up of the newborn.

The second type of congenital melanoma is primary congenital melanoma, which may develop *de novo* or in association with a congenital nevus.[1,2] In our experience, congenital melanoma has a predilection for the scalp.[1] The third type of congenital melanoma is that which develops in large/giant CMN with or without neurocutaneous melanosis; this type likely constitutes the largest group of congenital melanoma. These melanomas are predominately dermal but might exhibit lentiginous and junctional nested arrangements of melanoma cells.

Leptomeningeal Melanoma Associated with Neurocutaneous Melanosis

Leptomeningeal melanoma can arise in approximately two thirds of patients with neurocutaneous melanosis.[36,37] They are intracranial in approximately 50% of patients and most commonly involve the frontal and temporal lobes. They are rapidly fatal in almost all patients (median age of death 3 years).

Summary

Identifying molecular markers which predict aggressive behavior of pediatric melanomas is critical for risk stratification and choosing appropriate treatment strategies. This is particularly true in view of the diversity of disease observed in this age group. Recent preliminary evidence suggests that alterations in *TERT* and aberrant telomere lengths might be particularly useful as prognostic markers in pediatric melanomas. These potential markers need to be validated in large patient cohorts, using long-term follow-up data, before their clinical implementation.

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Abbreviations Key

TERT	telomerase reverse transcriptase
FISH	fluorescence <i>in situ</i> hybridization
CGH	comparative genomic hybridization
CMN	congenital melanocytic nevi
PI3K	phosphatidylinositol 3-kinase

References

1. Barnhill RL, Flotte TJ, Fleischli M, et al. Cutaneous melanoma and atypical Spitz tumors in childhood. *Cancer* 1995;76(10):1833–1845. [PubMed: 8625056]
2. Barnhill RL, Spatz A. Congenital melanocytic nevi and associated neoplasms, congenital and childhood melanoma In: Barnhill RL, Piepkorn M, Busam KJ Pathology of melanocytic nevi and melanoma Berlin Heidelberg: Springer-Verlag; 2014 p 155–203.

3. Berk DR, LaBuz E, Dadras SS, et al. Melanoma and melanocytic tumors of uncertain malignant potential in children, adolescents and young adults--the Stanford experience 1995–2008. *Pediatr Dermatol* 2010;27(3):244–254. [PubMed: 20403119]
4. Spatz A, Ruiter D, Hardmeier T, et al. Melanoma in childhood: an EORTC-MCG multicenter study on the clinico-pathological aspects. *Int J Cancer* 1996;68(3):317–324. [PubMed: 8903473]
5. Han D, Zager JS, Han G, et al. The unique clinical characteristics of melanoma diagnosed in children. *Ann Surg Oncol* 2012;19(12):3888–3895. [PubMed: 22864798]
6. Livestro DP, Kaine EM, Michaelson JS, et al. Melanoma in the young: differences and similarities with adult melanoma: a case-matched controlled analysis. *Cancer* 2007;110(3):614–624. [PubMed: 17577228]
7. Pol-Rodriguez M, Lee S, Silvers DN, et al. Influence of age on survival in childhood spitzoid melanomas. *Cancer* 2007;109(8):1579–1583. [PubMed: 17326059]
8. Averbook BJ, Lee SJ, Delman KA, et al. Pediatric melanoma: analysis of an international registry. *Cancer* 2013;119(22):4012–4019. [PubMed: 24022819]
9. Moore-Olufemi S, Herzog C, Warneke C, et al. Outcomes in pediatric melanoma: comparing prepubertal to adolescent pediatric patients. *Ann Surg* 2011;253(6):1211–1215. [PubMed: 21451389]
10. Lange JR, Palis BE, Chang DC, et al. Melanoma in children and teenagers: an analysis of patients from the National Cancer Data Base. *J Clin Oncol* 2007;25(11):1363–1368. [PubMed: 17416855]
11. Lewis KG. Trends in pediatric melanoma mortality in the United States, 1968 through 2004. *Dermatol Surg* 2008;34(2):152–159. [PubMed: 18093205]
12. Berg P, Lindelof B. Differences in malignant melanoma between children and adolescents. A 35-year epidemiological study. *Arch Dermatol* 1997;133(3):295–297. [PubMed: 9080889]
13. Jen M, Murphy M, Grant-Kels JM. Childhood melanoma. *Clin Dermatol* 2009;27(6):529–536. [PubMed: 19880040]
14. Lu C, Zhang J, Nagahawatte P, et al. The genomic landscape of childhood and adolescent melanoma. *J Invest Dermatol* 2015;135(3):816–823. [PubMed: 25268584]
15. Lee S, Barnhill RL, Dummer R, et al. TERT Promoter Mutations Are Predictive of Aggressive Clinical Behavior in Patients with Spitzoid Melanocytic Neoplasms. *Sci Rep* 2015;5:11200. [PubMed: 26061100]
16. Fan Y, Lee S, Wu G, et al. Telomerase Expression by Aberrant Methylation of the TERT Promoter in Melanoma Arising in Giant Congenital Nevi. *J Invest Dermatol* 2016;136(1):339–342. [PubMed: 26763461]
17. Seynnaeve B, Lee S, Borah S, et al. Genetic and Epigenetic Alterations of TERT Are Associated with Inferior Outcome in Adolescent and Young Adult Patients with Melanoma. *Sci Rep* 2017;7:45704. [PubMed: 28378855]
18. Busam KJ, Murali R, Pulitzer M, et al. Atypical spitzoid melanocytic tumors with positive sentinel lymph nodes in children and teenagers, and comparison with histologically unambiguous and lethal melanomas. *Am J Surg Pathol* 2009;33(9):1386–1395. [PubMed: 19609204]
19. Hung T, Piris A, Lobo A, et al. Sentinel lymph node metastasis is not predictive of poor outcome in patients with problematic spitzoid melanocytic tumors. *Hum Pathol* 2013;44(1):87–94. [PubMed: 22939951]
20. Kinsler VA, Thomas AC, Ishida M, et al. Multiple Congenital Melanocytic Nevi and Neurocutaneous Melanosis Are Caused by Postzygotic Mutations in Codon 61 of NRAS. *J Invest Dermatol* 2013;133(9):2229–2236. [PubMed: 23392294]
21. Prieto-Granada CN, Lezcano C, Scolyer RA, et al. Lethal melanoma in children: a clinicopathological study of 12 cases. *Pathology* 2016;48(7):705–711. [PubMed: 27956274]
22. Paradela S, Fonseca E, Pita S, et al. Spitzoid melanoma in children: clinicopathological study and application of immunohistochemistry as an adjunct diagnostic tool. *J Cutan Pathol* 2009;36(7):740–752. [PubMed: 19032380]
23. Leech SN, Bell H, Leonard N, et al. Neonatal giant congenital nevi with proliferative nodules: a clinicopathologic study and literature review of neonatal melanoma. *Arch Dermatol* 2004;140(1):83–88. [PubMed: 14732664]

24. Vergier B, Laharanne E, Prochazkova-Carlotti M, et al. Proliferative Nodules vs Melanoma Arising in Giant Congenital Melanocytic Nevi During Childhood. *JAMA Dermatol* 2016;152(10):1147–1151. [PubMed: 27486690]
25. Fisher KR, Maize JC Jr., Maize JC Sr. Histologic features of scalp melanocytic nevi. *J Am Acad Dermatol* 2013;68(3):466–472. [PubMed: 23267721]
26. Fabrizi G, Pagliarello C, Parente P, et al. Atypical nevi of the scalp in adolescents. *J Cutan Pathol* 2007;34(5):365–369. [PubMed: 17448189]
27. Clark WH Jr., Hood AF, Tucker MA, et al. Atypical melanocytic nevi of the genital type with a discussion of reciprocal parenchymal-stromal interactions in the biology of neoplasia. *Hum Pathol* 1998;29(1 Suppl 1):S1–24. [PubMed: 9445124]
28. Pinto A, McLaren SH, Poppas DP, et al. Genital melanocytic nevus arising in a background of lichen sclerosis in a 7-year-old female: the diagnostic pitfall with malignant melanoma. A literature review. *Am J Dermatopathol* 2012;34(8):838–843. [PubMed: 22699866]
29. Thiagalingam S, Johnson MM, Colby KA, et al. Juvenile conjunctival nevus: clinicopathologic analysis of 33 cases. *Am J Surg Pathol* 2008;32(3):399–406. [PubMed: 18300811]
30. Evans MJ, Gray ES, Blessing K. Histopathological features of acral melanocytic nevi in children: study of 21 cases. *Pediatr Dev Pathol* 1998;1(5):388–392. [PubMed: 9688763]
31. Massi G. Melanocytic nevi simulant of melanoma with medicolegal relevance. *Virchows Arch* 2007;451(3):623–647. [PubMed: 17653760]
32. Krengel S, Hauschild A, Schafer T. Melanoma risk in congenital melanocytic naevi: a systematic review. *Br J Dermatol* 2006;155(1):1–8.
33. Yun SJ, Kwon OS, Han JH, et al. Clinical characteristics and risk of melanoma development from giant congenital melanocytic naevi in Korea: a nationwide retrospective study. *Br J Dermatol* 2012;166(1):115–123. [PubMed: 21923752]
34. Greene MH, Clark WH Jr., Tucker MA, et al. High risk of malignant melanoma in melanoma-prone families with dysplastic nevi. *Ann Intern Med* 1985;102(4):458–465. [PubMed: 3977193]
35. Lin J, Hocker TL, Singh M, et al. Genetics of melanoma predisposition. *Br J Dermatol* 2008;159(2):286–291. [PubMed: 18547303]
36. Kadonaga JN, Frieden IJ. Neurocutaneous melanosis: definition and review of the literature. *J Am Acad Dermatol* 1991;24(5 Pt 1):747–755. [PubMed: 1869648]
37. DeDavid M, Orlow SJ, Provost N, et al. Neurocutaneous melanosis: clinical features of large congenital melanocytic nevi in patients with manifest central nervous system melanosis. *J Am Acad Dermatol* 1996;35(4):529–538. [PubMed: 8859278]
38. Kraemer KH, Lee MM, Andrews AD, et al. The role of sunlight and DNA repair in melanoma and nonmelanoma skin cancer. The xeroderma pigmentosum paradigm. *Arch Dermatol* 1994;130(8):1018–1021. [PubMed: 8053698]
39. Ruiz-Maldonado R, Orozco-Covarrubias ML. Malignant melanoma in children. A review. *Arch Dermatol* 1997;133(3):363–371. [PubMed: 9080898]
40. Downard CD, Rapkin LB, Gow KW. Melanoma in children and adolescents. *Surg Oncol* 2007;16(3):215–220. [PubMed: 17976978]
41. Pappo AS. Melanoma in children and adolescents. *Eur J Cancer* 2003;39(18):2651–2661. [PubMed: 14642927]
42. Chudnovsky Y, Adams AE, Robbins PB, et al. Use of human tissue to assess the oncogenic activity of melanoma-associated mutations. *Nat Genet* 2005;37(7):745–749. [PubMed: 15951821]
43. Gray-Schopfer VC, Cheong SC, Chong H, et al. Cellular senescence in naevi and immortalisation in melanoma: a role for p16? *Br J Cancer* 2006;95(4):496–505. [PubMed: 16880792]
44. Shain AH, Yeh I, Kovalyshyn I, et al. The Genetic Evolution of Melanoma from Precursor Lesions. *N Engl J Med* 2015;373(20):1926–1936. [PubMed: 26559571]
45. Cancer Genome Atlas Network. Genomic Classification of Cutaneous Melanoma. *Cell* 2015;161(7):1681–1696. [PubMed: 26091043]
46. Omholt K, Platz A, Kanter L, et al. NRAS and BRAF mutations arise early during melanoma pathogenesis and are preserved throughout tumor progression. *Clin Cancer Res* 2003;9(17):6483–6488. [PubMed: 14695152]

47. Sullivan RJ, Flaherty K. MAP kinase signaling and inhibition in melanoma. *Oncogene* 2013;32(19):2373–2379. [PubMed: 22945644]
48. Tsao H, Goel V, Wu H, et al. Genetic interaction between NRAS and BRAF mutations and PTEN/MMAC1 inactivation in melanoma. *J Invest Dermatol* 2004;122(2):337–341. [PubMed: 15009714]
49. Haluska FG, Tsao H, Wu H, et al. Genetic alterations in signaling pathways in melanoma. *Clin Cancer Res* 2006;12(7 Pt 2):2301s–2307s. [PubMed: 16609049]
50. Dankort D, Curley DP, Carlidge RA, et al. Braf(V600E) cooperates with Pten loss to induce metastatic melanoma. *Nat Genet* 2009;41(5):544–552. [PubMed: 19282848]
51. Rodriguez-Viciana P, Warne PH, Vanhaesebroeck B, et al. Activation of phosphoinositide 3-kinase by interaction with Ras and by point mutation. *EMBO J* 1996;15(10):2442–2451. [PubMed: 8665852]
52. Wiesner T, He J, Yelensky R, et al. Kinase fusions are frequent in Spitz tumours and spitzoid melanomas. *Nature communications* 2014;5:3116.
53. Wu G, Barnhill RL, Lee S, et al. The landscape of fusion transcripts in spitzoid melanoma and biologically indeterminate spitzoid tumors by RNA sequencing. *Mod Pathol* 2016.
54. Michaloglou C, Vredeveld LC, Soengas MS, et al. BRAFE600-associated senescence-like cell cycle arrest of human naevi. *Nature* 2005;436(7051):720–724. [PubMed: 16079850]
55. Castellano M, Pollock PM, Walters MK, et al. CDKN2A/p16 is inactivated in most melanoma cell lines. *Cancer Res* 1997;57(21):4868–4875. [PubMed: 9354451]
56. Blackburn EH. Structure and function of telomeres. *Nature* 1991;350(6319):569–573. [PubMed: 1708110]
57. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell* 2011;144(5):646–674. [PubMed: 21376230]
58. Meyerson M, Counter CM, Eaton EN, et al. hEST2, the putative human telomerase catalytic subunit gene, is up-regulated in tumor cells and during immortalization. *Cell* 1997;90(4):785–795. [PubMed: 9288757]
59. Yi X, Shay JW, Wright WE. Quantitation of telomerase components and hTERT mRNA splicing patterns in immortal human cells. *Nucleic Acids Res* 2001;29(23):4818–4825. [PubMed: 11726691]
60. Horn S, Figl A, Rachakonda PS, et al. TERT promoter mutations in familial and sporadic melanoma. *Science* 2013;339(6122):959–961. [PubMed: 23348503]
61. Huang FW, Hodis E, Xu MJ, et al. Highly recurrent TERT promoter mutations in human melanoma. *Science* 2013;339(6122):957–959. [PubMed: 23348506]
62. Barthel FP, Wei W, Tang M, et al. Systematic analysis of telomere length and somatic alterations in 31 cancer types. *Nat Genet* 2017;49(3):349–357. [PubMed: 28135248]
63. Strouse JJ, Fears TR, Tucker MA, et al. Pediatric melanoma: risk factor and survival analysis of the surveillance, epidemiology and end results database. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2005;23(21):4735–4741. [PubMed: 16034049]
64. Whiteman DC, Valery P, McWhirter W, et al. Risk factors for childhood melanoma in Queensland, Australia. *Int J Cancer* 1997;70(1):26–31. [PubMed: 8985086]
65. Landi MT, Bauer J, Pfeiffer RM, et al. MC1R germline variants confer risk for BRAF-mutant melanoma. *Science* 2006;313(5786):521–522. [PubMed: 16809487]
66. Berg P, Wennberg AM, Tuominen R, et al. Germline CDKN2A mutations are rare in child and adolescent cutaneous melanoma. *Melanoma Res* 2004;14(4):251–255. [PubMed: 15305154]
67. Tosti A, Piraccini BM, Cagalli A, et al. In situ melanoma of the nail unit in children: report of two cases in fair-skinned Caucasian children. *Pediatr Dermatol* 2012;29(1):79–83. [PubMed: 21575049]
68. Hodis E, Watson IR, Kryukov GV, et al. A landscape of driver mutations in melanoma. *Cell* 2012;150(2):251–263. [PubMed: 22817889]
69. Schmid-Wendtner MH, Berking C, Baumert J, et al. Cutaneous melanoma in childhood and adolescence: an analysis of 36 patients. *J Am Acad Dermatol* 2002;46(6):874–879. [PubMed: 12063484]

70. Jafarian F, Powell J, Kokta V, et al. Malignant melanoma in childhood and adolescence: report of 13 cases. *J Am Acad Dermatol* 2005;53(5):816–822. [PubMed: 16243130]
71. Stanelle EJ, Busam KJ, Rich BS, et al. Early-stage non-Spitzoid cutaneous melanoma in patients younger than 22 years of age at diagnosis: long-term follow-up and survival analysis. *J Pediatr Surg* 2015;50(6):1019–1023. [PubMed: 25819019]
72. Balch CM, Gershenwald JE, Soong SJ, et al. Final version of 2009 AJCC melanoma staging and classification. *J Clin Oncol* 2009;27(36):6199–6206. [PubMed: 19917835]
73. Lallas A, Kyrgidis A, Ferrara G, et al. Atypical Spitz tumours and sentinel lymph node biopsy: a systematic review. *Lancet Oncol* 2014;15(4):e178–183. [PubMed: 24694641]
74. Barnhill RL, Argenyi ZB, From L, et al. Atypical Spitz nevi/tumors: lack of consensus for diagnosis, discrimination from melanoma, and prediction of outcome. *Hum Pathol* 1999;30(5):513–520. [PubMed: 10333219]
75. Barnhill RL. The Spitzoid lesion: rethinking Spitz tumors, atypical variants, ‘Spitzoid melanoma’ and risk assessment. *Mod Pathol* Feb19 Suppl 2006;2 SRC - GoogleScholar:S21–33.
76. Zhao G, Lee KC, Peacock S, et al. The utilization of spitz-related nomenclature in the histological interpretation of cutaneous melanocytic lesions by practicing pathologists: results from the M-Path study. *J Cutan Pathol* 2017;44(1):5–14. [PubMed: 27686456]
77. Ferrari A, Bono A, Baldi M, et al. Does melanoma behave differently in younger children than in adults? A retrospective study of 33 cases of childhood melanoma from a single institution. *Pediatrics* 2005;115(3):649–654. [PubMed: 15741367]
78. Cordoro KM, Gupta D, Frieden IJ, et al. Pediatric melanoma: results of a large cohort study and proposal for modified ABCD detection criteria for children. *J Am Acad Dermatol* 2013;68(6):913–925. [PubMed: 23395590]
79. Requena C, Botella R, Nagore E, et al. Characteristics of spitzoid melanoma and clues for differential diagnosis with spitz nevus. *Am J Dermatopathol* 2012;34(5):478–486. [PubMed: 22257900]
80. Yeh I, Tee MK, Botton T, et al. NTRK3 kinase fusions in Spitz tumours. *J Pathol* 2016;240(3):282–290. [PubMed: 27477320]
81. Yeh I, Botton T, Talevich E, et al. Activating MET kinase rearrangements in melanoma and Spitz tumours. *Nature communications* 2015;6:7174.
82. Wiesner T, Kutzner H, Cerroni L, et al. Genomic aberrations in spitzoid melanocytic tumours and their implications for diagnosis, prognosis and therapy. *Pathology* 2016;48(2):113–131. [PubMed: 27020384]
83. Wiesner T, Murali R, Fried I, et al. A distinct subset of atypical Spitz tumors is characterized by BRAF mutation and loss of BAP1 expression. *Am J Surg Pathol* 2012;36(6):818–830. [PubMed: 22367297]
84. Ali L, Helm T, Cheney R, et al. Correlating array comparative genomic hybridization findings with histology and outcome in spitzoid melanocytic neoplasms. *Int J Clin Exp Pathol* 2010;3(6):593–599. [PubMed: 20661407]
85. Bastian BC, Wesselmann U, Pinkel D, et al. Molecular cytogenetic analysis of Spitz nevi shows clear differences to melanoma. *J Invest Dermatol* 1999;113(6):1065–1069. [PubMed: 10594753]
86. Mehregan AH, Mehregan DA. Malignant melanoma in childhood. *Cancer* 1993;71(12):4096–4103. [PubMed: 8508375]
87. Spatz A, Calonje E, Handfield-Jones S, et al. Spitz tumors in children: a grading system for risk stratification. *Arch Dermatol* 1999;135(3):282–285. [PubMed: 10086449]
88. Soo JK, Mackenzie Ross AD, Kallenberg DM, et al. Malignancy without immortality? Cellular immortalization as a possible late event in melanoma progression. *Pigment Cell Melanoma Res* 2011;24(3):490–503. [PubMed: 21418545]
89. Artandi SE, DePinho RA. Telomeres and telomerase in cancer. *Carcinogenesis* 2010;31(1):9–18. [PubMed: 19887512]
90. Crowley NJ, Seigler HF. Late recurrence of malignant melanoma. Analysis of 168 patients. *Ann Surg* 1990;212(2):173–177. [PubMed: 2375648]

91. Crotty KA, McCarthy SW, Palmer AA, et al. Malignant melanoma in childhood: a clinicopathologic study of 13 cases and comparison with Spitz nevi. *World J Surg* 1992;16(2):179–185. [PubMed: 1561797]
92. Marghoob AA, Schoenbach SP, Kopf AW, et al. Large congenital melanocytic nevi and the risk for the development of malignant melanoma. A prospective study. *Arch Dermatol* 1996;132(2):170–175. [PubMed: 8629825]
93. Kregel S, Scope A, Dusza SW, et al. New recommendations for the categorization of cutaneous features of congenital melanocytic nevi. *J Am Acad Dermatol* 2013;68(3):441–451. [PubMed: 22982004]
94. Neuhold JC, Friesenhahn J, Gerdes N, et al. Case reports of fatal or metastasizing melanoma in children and adolescents: a systematic analysis of the literature. *Pediatr Dermatol* 2015;32(1):13–22. [PubMed: 25487565]
95. Charbel C, Fontaine RH, Malouf GG, et al. NRAS mutation is the sole recurrent somatic mutation in large congenital melanocytic nevi. *J Invest Dermatol* 2014;134(4):1067–1074. [PubMed: 24129063]
96. Lee S, Borah S, Bahrami A. Detection of Aberrant TERT Promoter Methylation by Combined Bisulfite Restriction Enzyme Analysis for Cancer Diagnosis. *The journal of Molecular Diagnostics* 2017.
97. Su A, Low L, Li X, et al. De novo congenital melanoma: analysis of 2 cases with array comparative genomic hybridization. *Am J Dermatopathol* 2014;36(11):915–919. [PubMed: 25051103]
98. Bastian BC, Xiong J, Frieden IJ, et al. Genetic changes in neoplasms arising in congenital melanocytic nevi: differences between nodular proliferations and melanomas. *Am J Pathol* 2002;161(4):1163–1169. [PubMed: 12368190]
99. Alexander A, Samlowski WE, Grossman D, et al. Metastatic melanoma in pregnancy: risk of transplacental metastases in the infant. *J Clin Oncol* 2003;21(11):2179–2186. [PubMed: 12775744]
100. Ball RA, Genest D, Sander M, et al. Congenital melanocytic nevi with placental infiltration by melanocytes: a benign condition that mimics metastatic melanoma. *Arch Dermatol* 1998;134(6):711–714. [PubMed: 9645640]

Figure 1.

Photomicrographs of hematoxylin and eosin-stained (H&E) sections and immunohistochemical stains for p16 and BRAF V600E in an adult subtype melanoma from a peri-adolescent patient.

Malignant melanoma, nodular type (A; H&E, scanning magnification), composed of confluent nests of epithelioid malignant melanocytes (B, H&E 20×). Immunohistochemical analysis shows loss of p16 expression (C) and positive staining for BRAF V600E (D) in the melanoma cells. The melanoma harbored a *BRAF*^{V600E} and a *TERT* promoter mutation.

Figure 2.

Photomicrographs of H&E stained sections and interphase fluorescence *in situ* hybridization (FISH) with *RET* and *ROS1* dual color, break-apart probe from patients with pediatric spitzoid melanocytic tumors.

A-C: Pediatric spitzoid melanoma with predominantly spindle cell features (A, H&E 4×; B, H&E 40×) harboring *RET* fusion. FISH image (C) shows split red and green signals, consistent with *RET* rearrangement.

D-F: Pediatric spitzoid melanocytic tumor with epithelioid and spindle cell features (D, H&E 4×; E, H&E 20×) harboring *ROS1* fusion. FISH image (F) shows separate red and green signals in several nuclei, consistent with *ROS1* rearrangement.

Figure 3.

Photomicrographs of H&E sections and TERT mRNA in situ hybridization (ISH) in a melanoma and in a proliferative nodule arising in association with giant congenital nevi.

A-C: Melanoma consisting of malignant small round cells in the dermis within a giant congenital nevus (A, H&E 4 $\times$; B, H&E 40 $\times$). TERT mRNA ISH shows numerous punctate intracellular signals (C), consistent with high TERT mRNA levels.

D-F: Proliferative nodule arising within a giant congenital nevus in deep soft tissue, characterized by cellular features, mitotic activity (D, H&E 2 $\times$; E, H&E 40 $\times$), and no significant levels of TERT mRNA detected by ISH (F).