UPDATE IN MELANOCYTIC LESIONS

Course Directors: J. Andrew Carlson and Philip E. LeBoit, Course Directors

Objectives:
Following this course, the attendee should be able to:
1. Discuss the molecular biology and critical aberrant genomic mechanisms that occur in the progression of melanoma. 2. Recognize the morphology and molecular pathology of some melanoma variants and borderline sub-types. 3. Discuss the newly available molecular tests and the novel treatment options for metastatic melanoma such as immunotherapy.

Description:
Dramatic progress has been made over the last 5 years increasing our knowledge of the molecular mechanisms of melanoma progression. These novel insights have lead to the development of diagnostic and prognostic laboratory tests, and combination therapies that are anticipated to markedly improve melanoma management and outcomes. For dermatopathologists, it is essential that they evaluate and incorporate new methods and technologies based on a sound knowledge of melanoma pathogenesis and assessment of genomic-pathologic correlations.

Faculty:
Iwei Yeh: Genetic evolution of melanoma
Klaus Busam: Update on desmoplastic melanoma
Armita Bahrami: "Maintaining the telomeres in melanoma"
Christopher Shea: New perspectives on dysplastic nevi
Janis M. Taube: PD-L1 in melanoma: pathological challenges and clinical implications
Philip E. LeBoit: “New tools and new problems: the triumphs and limitations of molecular melanocytology”

SEE ATTACHED HANDOUTS SUBMITTED BY THE SPEAKERS
Genetic Evolution of Melanoma
Iwei Yeh MD, PhD
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Evolution describes the change in heritable characteristics of a biological population over successive generations. Natural selection results in survival of the fittest and shapes evolution. The study of the genetic relationships of organisms and their probable evolutionary paths has shaped our understanding of evolution and is incorporated into modern taxonomy.

The clonal evolution of tumor cell populations was first proposed in 1976\(^1\). Cancer arises from a single cell of origin through the accumulation of multiple genetic mutations. As each individual cancer is an evolutionary dead end, the study of cancer genetics is that of recurring evolutionary pathways. In many cases the cell type from which a cancer arises narrowly constrains the range of mutations that result in transformation. In addition, oncogenic mutations affect phenotypic characteristics of tumors, resulting in an amazing correspondence between our histopathologic taxonomy of disease and their genetic basis.

Tumor initiation refers to the changes to a normal cell that enables tumor formation. \(\text{BRAF}^{V600E}\) is the best-studied initiating oncogene in melanocytic neoplasia. It has been identified in \(~90\%\) of common acquired nevi and demonstrated to be present in every melanocyte of such nevi\(^2,3\). Targeted sequencing of common acquired nevi for other melanoma oncogenes has not identified any other genetic alterations, suggesting that \(\text{BRAF}\) mutation alone is sufficient for transformation. To date there are 14 probable initiating oncogenes identified in melanocytic nevi that activate the mitogen-activated protein kinase pathway and occur in a mutually exclusive fashion\(^4-12\). Initiating oncogenes correspond with the histopathologic subtype of the nevus and those that give rise to the same subtype of nevus cluster within signaling pathways.

Melanoma occurs due to additional genetic alterations that support the acquisition of specific hallmarks of cancer\(^13\) resulting in unrestrained growth, metastasis and immune evasion. In some cases, populations of melanocytes from different stages of genetic progression can be distinguished based on histopathologic features within the same tumor. One example is the loss of BAP1 in a common nevus that results in clonal expansion of melanocytes with spitzoid cytomorphology\(^14-16\). Understanding the genetic underpinnings of this tumor suggests a revision of our taxonomy of melanocytic neoplasia, removing these \(\text{BRAF}\) mutant tumors from the Spitz category of tumors and renaming them BAP1-inactivated spitzoid nevi\(^16\).
In a recent study, evolutionary pathways of melanoma were inferred from molecular characterization of melanoma and their precursor lesions. This study demonstrated that melanocytic tumors with “borderline” histopathologic diagnoses and increased interobserver disagreement harbored a wide range of secondary oncogenic alterations. Additional genetic alterations were observed in bona fide melanomas. This finding conclusively demonstrates that there are genetically intermediate tumors and the wide array of genetic alterations in these tumors may account for their diagnostic difficulty.

Metastatic melanoma represents a population of related subclones. Multiple subclones within a primary tumor may independently metastasize. Genetic mechanisms of treatment resistance include loss of function JAK mutations conferring resistance to PD-1 blockade and NRAS mutations conferring resistance to BRAF inhibitors.
References:
UPDATE ON DESSMOPLASTIC MELANOMA
Klaus J Busam, MD
Memorial Sloan Kettering Cancer Center, New York, NY

What has been known for a while:

Desmoplastic melanoma (DM) is a rare variant of melanoma characterized by the association of invasive melanoma with a dense fibrous matrix. It typically affects elderly Caucasians, most often in the head and neck region. In contrast to the majority of conventional melanomas, DM, especially in its pure pauci-cellular phenotype, is less likely to involve regional lymph nodes. Among thick invasive tumors, the presence of a pure desmoplastic histology is also associated with better disease-specific survival. DM also remains a diagnostic challenge as it can be confused with a range of benign and malignant tumors.

What we have learned over the past 5 years:

1. Neurofibromin plays a role in desmoplastic melanoma
   - Mutations in NF1 are common in desmoplastic melanoma

2. Microscopic Findings
   - Lymphocytic aggregates can occur in desmoplastic nevi

3. Immunohistochemistry
   - Sox 10 is a sensitive marker for the detection of DM, but with limited specificity
   - The CD34 fingerprint pattern is not specific for neurofibromas
   - DM can express p16

4. Molecular Tests for Diagnosis
   - A positive cytogenetic test can help distinguish DM from some nevi
   - Mutation analysis can in selected cases distinguish DM from nevi

References:
Maintaining Telomeres in Melanoma
Armita Bahrami, MD
St. Jude Children’s Research Hospital, Memphis, TN

This talk discusses the applications of DNA-level TERT alterations as a potential prognostic tool in melanocytic neoplasms of uncertain malignant potential.

Somatic cells have limited replicative capacity because of progressive telomere shortening that occurs during iterative cycles of replication. 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. Telomere lengths are maintained in 85%–90% of cancer cell lines and tissues through expression of the telomerase reverse transcriptase (TERT) oncogene. TERT, which is normally silenced in somatic cells, encodes the catalytic subunit of telomerase, the telomere-lengthening enzyme. In many of the remaining cancers which do not express TERT, telomere length maintenance is achieved by a telomerase-independent mechanism called alternative lengthening of telomeres (ALT).

To catalogue the relative predominance of different types of DNA-level TERT alterations in melanoma, we screened 70 metastatic melanoma samples from adult patients for three commonly-occurring alterations: mutation, hypermethylation, and structural rearrangement. 90% of these samples harbored TERT promoter mutation and/or hypermethylation, and the remaining samples often harbored TERT structural rearrangements. Promoter mutation and rearrangement occurred in a mutually exclusive fashion. On the other hand, evidence for activation of the ALT mechanism was rarely observed. The DNA-level TERT alterations correlated with high TERT mRNA expression levels (Lee S, et al. unpublished data). Thus, the combination of TERT promoter mutations, hypermethylation, and rearrangements is likely to be an effective diagnostic proxy for TERT mRNA levels.

Despite the inherently complex genomic profile of malignant melanocytes, alterations in a defined set of molecular pathways seem to drive melanoma tumorigenesis. These critical pathways are dysregulated stepwise, often in the following order: i) activation of the RAS/RAF/MAP kinase pathway; ii) activation of telomerase; iii) inhibition of the p16/Rb pathway, and PTEN alterations. This model depicts the evolution of invasive melanoma in adult patients. However, the same series of steps may not be followed by their pediatric counterparts, which are histologically similar but biologically distinct. In a study of spitzoid neoplasms, a subset of pediatric melanomas were observed to acquire p16 biallelic loss yet did not activate telomerase; these cases followed a benign clinical course. The favorable outcome commonly observed in these otherwise morphologically malignant pediatric tumors may be explained by the fact that they lacked a means of maintaining telomere length, and therefore could not sustain continued proliferation. DNA-level alterations of TERT may thus also serve as a useful diagnostic marker, in conjunction with other parameters, for pediatric melanocytic tumors of an ambiguous nature.

References:
DYSPLASTIC NEVUS UPDATE: ISDP 2017, Orlando, FL

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Eugene J. Van Scott Professor in Dermatology
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Dysplastic nevi (DN, Clark nevi) remain a source of concern because of: (a) clinical and pathologic morphological features that sometimes overlap those of melanoma; (b) demonstrated role as a risk factor for development of melanoma; (c) controversial role as a potential precursor at risk for progression to melanoma; and (d) medico-legal implications of these issues.

For these reasons, DN are commonly biopsied or excised. The goals of removing atypical / dysplastic nevi may include: 1. Accurate pathologic diagnosis and, in particular, ruling in or ruling out the alternative possibility of malignant melanoma; 2. Reducing the chance of recurrence after partial biopsy; 3. Correlating clinical and pathologic features of a given patient’s pigmented lesion(s); and 4. Rarely, preventing progression of an atypical nevus to outright melanoma. Ideally, in order to achieve any or all of these goals, when an atypical / dysplastic nevus is biopsied it should be conservatively but completely removed and submitted for pathologic study. The resulting specimens may measure < 6 mm in greatest dimension and often will have been obtained by use of shave or punch techniques. Only a small percentage of the total surgical margin in these small specimens can be assessed microscopically. A positive margin can usually be declared with confidence, but any statement that margins are negative will reflect probabilities rather than strict facts.

While wide regional and personal variations exist in clinical practice, recent literature suggests that many clinicians take into account both the grade of histopathologic atypia and the apparent margin status when determining whether to perform a re-excision on previously biopsied atypical / dysplastic nevi. It is recommended that pathologists be clear and consistent in their terminology regarding margin status, as well as in any recommendations regarding re-excision. Pathologists can best serve patients by engaging in an open discussion about referring clinicians’ goals, preferences, and expectations regarding reporting of margins in atypical / dysplastic nevi. Emerging literature supports the conclusion that the great majority of DN, if incompletely removed, do not pose a significant risk of progressing to melanoma. This suggests that a number of DN may not require removal.

MPATH-Dx is a recently described pathologic reporting schema that attempts to map diagnosis and treatment of pigmented lesions, and that may help to categorize melanocytic lesions, including DN, according to a hierarchy of suggested treatment.
References:
Duffy KL, Mann DJ, Petronic-Rosic V, Shea CR. Arch Dermatol 2012; 148 (2): 259-60


Fleming NH, Egbert BA, Kim J, Swetter SM. Reexamining the threshold for reexcision of histologically transected dysplastic nevi. JAMA Dermatol 2016: 152 (12): 1327-34


**PD-L1 in melanoma: pathological challenges and clinical implications**
Janis M. Taube, MD
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**Abstract:** PD-L1 expression in the pre-treatment tumor microenvironment has been shown to enrich for response to anti-PD-1 and anti–PD-L1. The predominant method for assessing PD-L1 in this setting has been PD-L1 immunohistochemistry, and there are recognized challenges in its assessment. In melanoma, PD-L1 expression often represents an adaptive immune resistance mechanism to surveilling host T-cells. As such, its expression may be focal and geographic, potentially leading to sampling error. Additionally, there are now a number of PD-L1 assays that are available, which presents challenges to pathology laboratories and pathologists charged with interpreting the different assays. Initial studies are emerging regarding the comparative performance of the commercially-available assays. The studies suggest that the differences in assay performance are a function of the assay conditions, rather than the antibodies themselves. Now that the initial comparisons will be performed, the next steps for the field will undoubtedly include attempts at assay harmonization and the development of laboratory derived tests. Other single markers beyond PD-L1 have been associated with response to anti-PD-1, such as CD8+ T-cell density and mutational load. Studies in the TCGA dataset highlight that a cytotoxic gene signature and PD-L1 expression are very closely related in melanoma, while mutational density is not directly related to an inflamed tumor phenotype. When these factors are prioritized for their impact on melanoma prognosis, an inflamed tumor phenotype is the most important factor, followed by mutational density. Future assays for prognostication and prediction of response to immunotherapies will undoubtedly be both multimodality and multiplexed.

**References:**