The Impact of Next-Generation Sequencing on Interobserver Agreement and Diagnostic Accuracy of Desmoplastic Melanocytic Neoplasms

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Introduction

Next-generation sequencing (NGS) is increasingly being utilized as an ancillary tool for diagnostically challenging melanocytic neoplasms (DMNs). Current ancillary testing used by pathologists to approach this diagnostic problem have significant limitations:

- Cytogenetic studies have shown that the sensitivity of FISH to identify desmoplastic melanomas (DMs) is less than 50%[1]
- PRAME immunohistochemistry (IHC) has only been shown to be positive in 35% of DMs[2,3]

In this study, we aim to assess whether NGS improves interobserver agreement and the diagnosis of diagnostically challenging DMNs.

Methods

Genomic Sequencing:
NGS was performed on 47 cases either at the time of clinical care (n = 23) or later for research purposes (n = 24). The following panels were used for sequencing: PGDx NGS Panel, Oncomine Precision Assay NGS Panel, Tempus xT Panel, Comprehensive Cancer NGS Panel, Fusion Plex Pan Solid Tumor v2 Sequencing Panel, and TERT promoter assay.

Survey Creation:
Two surveys were created with the 47 de-identified cases and presented to 20 expert dermatopathologists in a random order:

- Survey 1 consisted of limited clinical information (age, sex, anatomic site, digital link to H&E slide) and given six possible diagnostic choices.
- Survey 2 included this information plus additional genomic sequencing data on various genetic alterations (missense, truncation, fusion, amplifications, whole gene deletions, etc.).

Statistical Analysis:
To interpret Fleiss κ values, the following inter-rater reliability categories were used: 0-0.20 as none to slight, 0.21-0.39 as fair, 0.40-0.59 as moderate, 0.60-0.79 as substantial, 0.80-1 as almost perfect agreement. $\chi 2$ tests to measure the significant difference between the first and second survey results were calculated. The significance level was set at $\alpha = 0.05$.

Results

Characteristics	Desmoplastic melanoma (DM)	Benign or intermediate grade desmoplastic tumor (BIDT)	P-Value
N	15	28	
Mean age (years)	69	41	**P < 0.00001**
Female:male ratio	11:4	17:11	P = 0.41
Location			
Head & neck	47% (7)	9% (3)	**P = 0.0078**
Trunk	33% (5)	36% (12)	P = 0.54
Extremities	20% (3)	46% (13)	P = 0.088

Table 1. Demographic Overview of Cases

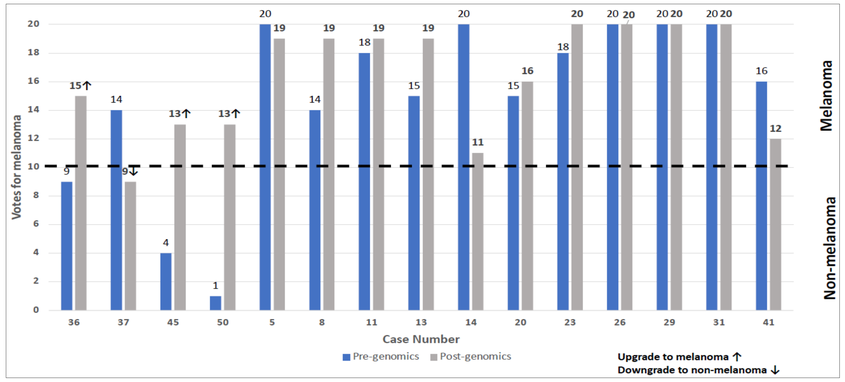

Figure 1. Changes in votes for malignant melanoma cases (n = 15), pre-genomics and post-genomics. All cases with at least 50% (≥ 10) of the votes (above the dotted line) indicate a majority diagnosis of melanoma. All cases with less than 50% (≤ 10) of the votes (below the dotted line) indicate a majority diagnosis of non-melanoma. The up arrows in cases 36, 45, and 50 indicate an upgrade in the diagnosis to melanoma if the majority diagnosis was originally non-melanoma (below the dotted line) and then changed to melanoma (above the dotted line) post-genomics. The down arrow in case 37 indicates a downgrade in the diagnosis to non-melanoma if the majority diagnosis was originally melanoma (above the dotted line) and then changed to non-melanoma (below the dotted line) post-genomics.

	Genetic Alterations
Genomic Changes Favoring a Diagnosis of Desmoplastic Melanoma (DM)	Frame shift, splice site or non-sense mutations involving *NF1, ARID2, CDKN2A or TP53*
	Activating missense mutations in BRAF + TERT-p hotspot mutation
	Activating missense mutations in NRAS + TERT-p hotspot mutation
Genetic Changes Favoring a Diagnosis of Benign and Intermediate Grade Desmoplastic Tumor (BIDT)	*Activating missense mutations in BRAF, NRAS or HRAS* in the absence of other pathogenic variants typical of melanoma tumor progression
	Activating Missense mutations in GNA11 or GNAQ in the absence of other pathogenic variants typical of malignant blue nevus tumor progression

Table 2. Genomic Alterations in Appropriately Upgraded and Downgraded Cases

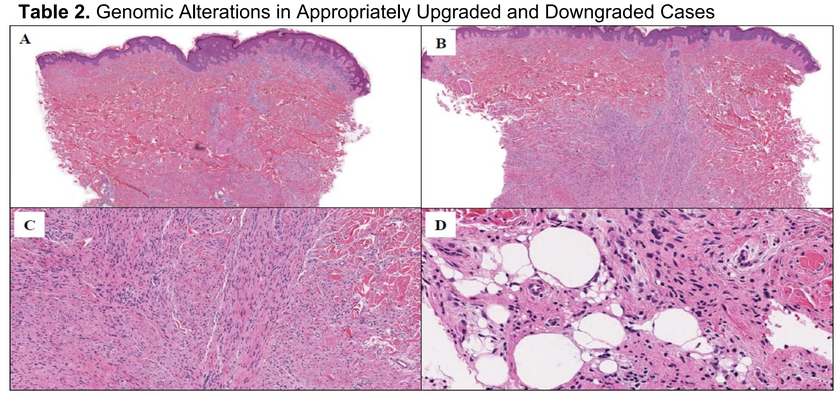

Figure 2. Case #36, a desmoplastic melanoma (DM) which resulted in distant metastasis. Low power magnification (A and B) demonstrates a combined phenotype with an atypical epithelioid component directly beneath the epidermis and an atypical spindle cell component with sclerotic stroma in the deeper dermis. Higher power magnification (C and D) demonstrates fascicles of hyperchromatic atypical spindle cells diving down into the dermis and extending into the subcutaneous fat.

Discussion

- Key genomic findings that correctly influenced participants to favor a diagnosis of DM included truncating mutations, splice site alterations or frameshift mutations involving NF1, TP53, ARID2, and CDKN2A.
- The presence of a BRAF or NRAS mutation in combination with a TERT promoter mutation was a useful finding favoring melanoma.
- When considering both the upgrades and downgrades in the DM cases pre- and post-genomics, there was a net improvement in the diagnostic accuracy of the DMs, showing that NGS has the potential to improve diagnostic accuracy in the assessment of desmoplastic melanocytic tumors.
- The degree of improvement will be most substantial among pathologists with some background and experience in bioinformatics and melanoma genetics.

References

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