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Immunohistochemical Expression of SATB2 in Malignant Melanomas

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Abstract: Accurate diagnosis of cancer of unknown primary (CUP) poses a significant daily challenge for pathologists, necessitating reliable immunohistochemical (IHC) markers. SATB2 is a transcription factor primarily expressed in colorectal neoplasms. This study investigates the IHC expression of SATB2 in malignant melanomas (MM). Using tissue microarrays (TMAs) from Aalborg University Hospital, Denmark, comprising 56 primary and 12 metastatic MMs, we evaluated SATB2 expression through *H*-scores. We found that 48% of MM cases expressed SATB2, predominantly with weak to moderate staining intensity. Although no significant difference was observed between primary and metastatic MMs, a higher median *H*-score was noted in metastatic lesions. The results highlight the potential diagnostic pitfall of SATB2 expression in MM and underline the need for careful interpretation.

Key Words: SATB2, melanoma, IHC, immunohistochemistry, CUP

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The accurate diagnosis of cancer of unknown primary (CUP) remains a significant challenge for pathologists. Identifying reliable immunohistochemical (IHC) markers for diagnostic IHC panels is crucial to determining metastatic tumors' origin.

SATB2 (Special AT-rich sequence-binding protein 2), is a transcription factor that has shown promising results in differentiating colorectal neoplasms from other malignancies. SATB2 is a nuclear marker expressed in specific tissues, including epithelium in the lower gastrointestinal tract, brain, lymphoid cells, and tubuli of the testis. In addition, SATB2 expression has been identified in neuroendocrine neoplasms of the lower GI and Merkel cell tumors.

As our understanding of this marker evolves, novel and unexpected expression patterns are observed. The authors of this study noted SATB2 expression in a subset of malignant melanomas (MM), a finding not previously discussed in the literature. To our knowledge, only 2 studies have been published presenting any IHC SATB2 expression in melanomas: One article by Szczepanski et al[1] found IHC SATB2 expression in 2/13 sarcomatoid MM. Another study by Gallagher et al[2] found SATB2 expression in MM with osteocartilaginous differentiation.

While CK20 and CDX2 are the most established markers for identifying colorectal adenocarcinomas, SATB2 has been recognized as an additional marker with potential utility especially concerning specificity.[3] However, SATB2 is not commonly used as a first-line marker for colorectal origin. Even though differential diagnosis between colon adenocarcinoma and malignant melanoma is not a frequent scenario, our initial observation of SATB2 expression in MM prompted further investigation. This study aims to elucidate the potential for misinterpretation of SATB2 expression in the diagnostic work-up of cancers of unknown primary (CUP) by examining the SATB2 IHC expression in a cohort of malignant melanomas using the most successfully applied antibody based on NordiQC proficiency runs for SATB2.

MATERIALS AND METHODS

Tissues

Tissue microarrays (TMAs) utilized in this study were selected from previous IHC optimization studies and included tissues from the archives of the Department of Pathology at Aalborg University Hospital, Denmark. All included tissue samples were fully anonymized upon inclusion. The tissues were processed and paraffin-embedded by standard operating procedures including fixation in 10% neutral buffered formalin for a period typically ranging from 24 to 72 hours. The TMAs comprised 56 primary MMs, 12 metastatic MMs, 4 dysplastic nevi, 2 compound nevi, and 2 dermal nevi. No MM with osteocartilaginous differentiation was available for this study.

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Immunohistochemistry

TMA sections were cut at 3 μm and stained on the Ventana Benchmark Ultra platform. All slides were deparaffinized and subsequently submitted to Heat-Induced Epitope Retrieval (HIER) for 64 minutes using Ultra Cell Conditioning Solution (ULTRA CC1, Ventana Medical Systems, USA). IHC was performed using a rabbit monoclonal antibody against SATB2 (clone EP281, dilution 1:100; Cell Marque, USA) with an incubation time of 32 minutes and OptiView DAB IHC Detection Kit (Ventana Medical Systems) as a visualization system. On-slide positive and negative tissue controls were utilized. Epithelial cells of colon mucosa exhibiting moderate to strong nuclear staining reaction were used as high expressers, weakly to moderately stained dispersed germ cells in the testis as low expressers, and smooth muscle cells in muscularis propria as negative tissue control. The SATB2 IHC assay had initially been developed and validated on a wide range of normal and neoplastic tissues.

H-scores

The *H*-score method was utilized to evaluate immunohistochemical staining intensity and extent in TMA cores. In brief, a pathologist (R.R.) estimated the percentage of neoplastic cells stained at different intensity levels categorized as negative, weak, moderate, or strong. *H*-scores were calculated as (1x% of cells stained weakly) +(2x% of cells stained moderately)+(3x% of cells stained strongly). This yielded a score ranging from 0 to 300. An *H*-score of 20 or above was considered as positive. Only nuclear SATB2 staining in neoplastic cells was included. Reactions in, for example, lymphocytes were not included.

Statistical Analysis and Figures

Data management and statistical analysis were accomplished in R studio. The *H*-scores were assessed using the Mann-Whitney *U* test for comparison between groups, while the proportions of positive cases were evaluated using a Fisher exact test. The plot was generated using the package ggplot2.

RESULTS

Seven tissue cores were excluded from analysis due to missing TMA cores, lack of neoplastic cells, or excessive cytoplasmic IHC reaction. All dermal, compound, and dysplastic nevi were negative for SATB2 (*H*-score 0 to < 5).

On the basis of an *H*-score cutoff of 20, 30/63 (48%) of MM were positive for SATB2. The *H*-score in positive cases ranged from 20 to 210 (median 45) indicating that most positive cases were weakly to moderately stained. One case of MM with spindle cell features showed a weak SATB2 reaction (*H*-score of 30). Examples of SATB2 staining are presented in Figure 1.

Among primary MM, 23 of 52 cases (44%) exhibited SATB2 positivity, compared with 7 of 11 cases (63%) in metastatic lesions (Fig. 2). However, this disparity did not achieve statistical significance ($P > 0.05$). The median *H*-score for SATB2 expression was 40 in primary MM and 60 in metastatic lesions. This difference was not found to be statistically significant.

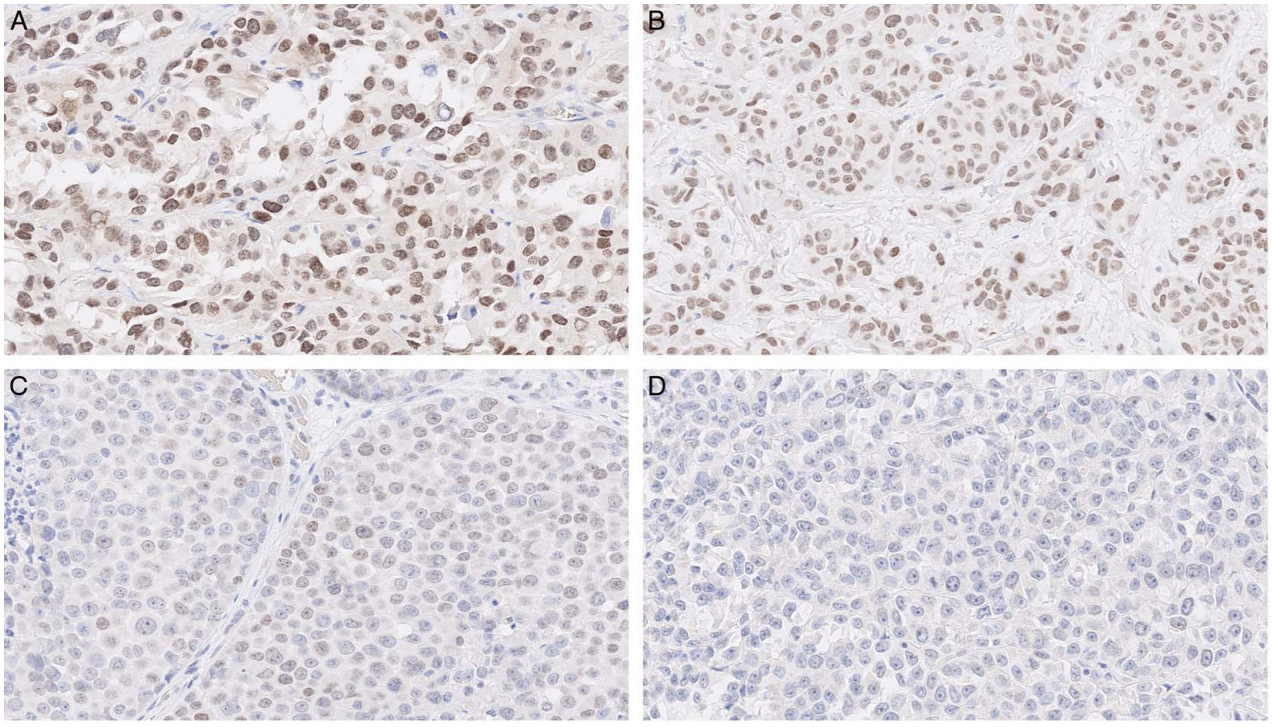

FIGURE 1. Nuclear staining for SATB2 in different malignant melanomas (MM). A–D, Moderate to strong, moderate, weak, and negative staining.

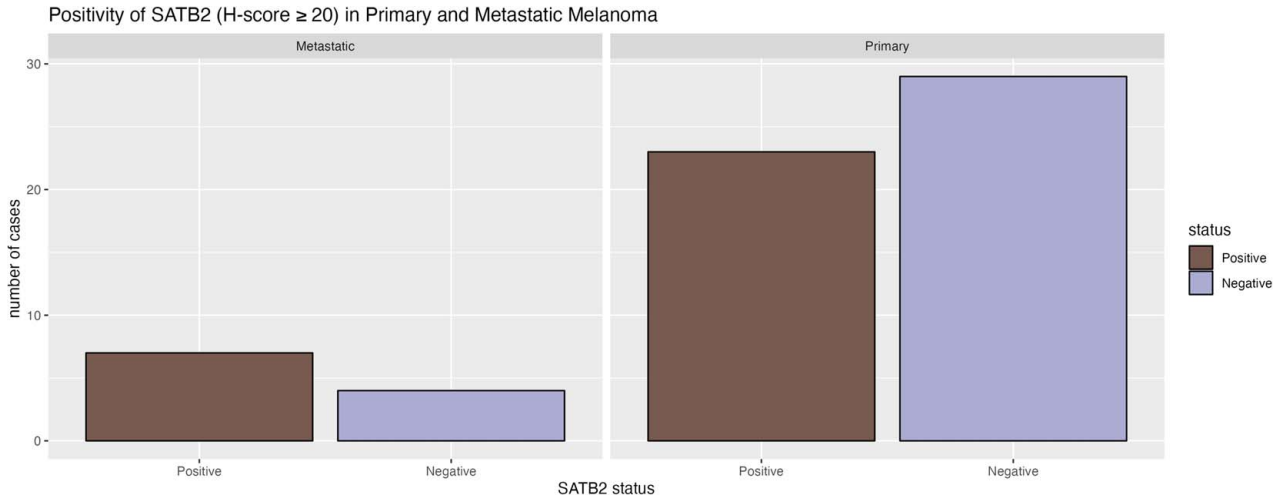

FIGURE 2. The number of SATB2 positive (*H*-score ≥ 20) and negative cases in primary and metastatic malignant melanoma.

DISCUSSION

In this study, we investigated the IHC expression of SATB2 in MM. We observed that 48% of MM cases expressed SATB2 with most cases showing weak to moderate staining reaction. We did not find any difference in SATB2 expression levels between primary MM and metastatic lesions, although there was a trend toward higher SATB2 intensity and extension.

While our findings provide insights into the potential pitfall of SATB2 expression in MM, our results should be interpreted within the limitations of the study. The experimental setup, including the relatively limited number of tissue samples, may have influenced our results. The inclusion of a larger and more diverse cohort could enhance the robustness of our findings—especially with a focus on uncommon histologic presentations.

One challenge encountered was defining a cutoff *H*-score for SATB2 positivity. We observed that varying the cutoff threshold resulted in significantly different proportions of positive cases: if we had selected *H*-score ≥ 10, 52% of MM would have been classified as positive. In contrast, with an *H*-score ≥ 50, only 22% would have been positive.

One article identified SATB1 expression in melanomas (85.1%), with SATB1 expression correlating to other established prognostic indicators (such as Breslow thickness and Clark level), as well as having independent prognostic value.[4] Since SATB1 and SATB2 share some DNA sequences, it raises the possibility that the primary antibodies for SATB2 may react with both SATB1 and SATB2. In the original study, a polyclonal antibody was used. However, the presented immunohistochemical images showed cytoplasmic staining rather than nuclear localization expected for a transcription factor. This suggests to us that the observed staining might be due to cross-reactivity with an unknown cytoplasmic molecule rather than the true nuclear expression of SATB1.

PubMed search only identified one article that reported weak SATB2 expression in 7.5% of MM.[5] The relative lack of literature concerning SATB2 expression in MM may, in part, be attributed to the widespread use of suboptimal SATB2 IHC protocols, as evident from the latest SATB2 run in the NordiQC external proficiency program, where a substantial proportion of submitted slides (58%) exhibited suboptimal staining.[6] The most prevalent feature of insufficient results assessed by NordiQC was characterized by weak or completely false negative staining results in cells/tissues expected to express SATB2 as colon adenocarcinomas and neuroendocrine carcinomas from the lower gastrointestinal tract. The widespread application of suboptimal IHC assays with low analytical sensitivity for SATB2 could potentially explain the negative or limited positivity in melanomas with low-level SATB2 expression.

In conclusion, our study documented SATB2 expression in a large proportion of MM—both in primary and metastatic lesions. Clinical pathologists must be aware of this potential diagnostic pitfall.

REFERENCES

1. Szczepanski JM, Siddiqui J, Patel RM, et al. Expression of SATB2 in primary cutaneous sarcomatoid neoplasms: a potential diagnostic pitfall. *Pathology*. 2023;55:350–354.
2. Gallagher SJ, Bailey T, Rawson RV, et al. Melanoma with osseous or chondroid differentiation: a report of eight cases including SATB2 expression and mutation analysis. *Pathology*. 2021;53:830–835.
3. Brettfeld SM, Ramos BD, Berry RS, et al. SATB2 versus cdx2: a battle royale for diagnostic supremacy in mucinous tumors. *Arch Pathol Lab Med*. 2019;143:1119–1125.
4. Chen H, Takahara M, Oba J, et al. Clinicopathologic and prognostic significance of SATB1 in cutaneous malignant melanoma. *J Dermatol Sci*. 2011;64:39–44.
5. Dum D, Kromm D, Lennartz M, et al. SATB2 expression in human tumors a tissue microarray study on more than 15,000 tumors. *Arch Pathol Lab Med*. 2023;147:451–464.
6. NordiQC. Accessed 13 June 2024. https://www.nordiqc.org/downloads/assessments/158_108.pdf