Use of a Novel Rabbit Monoclonal Phospho-Histone H3 (Ser10) versus H&E Mitotic Count in Melanoma

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Introduction

Mitotic count is a known prognostic indicator in cutaneous melanoma.[1] The traditional hematoxylin and eosin (H&E) manner of counting mitotic figures (MFs) using a 40X objective is time consuming and prone to inter- and intra-observer variability.[2,3] MFs can also be difficult to assess in H&E-stained sections when poor fixation is observed.[4] Ser10 on histone H3 is phosphorylated in association with mitotic chromatin condensation in late G2 and M phase of the cell cycle and thus phospho-histone H3 (pHH3) can distinguish mitotic from apoptotic nuclei.[5] To date, a pHH3 polyclonal antibody has seemingly been a reliable marker for mitotic counting in different types of tumors.[1-4,6-8] Recently, a new commercially available rabbit monoclonal mitotic marker Ser10 pHH3 has been developed and was shown to be more specific than the widely used polyclonal pHH3.[5] In certain melanoma cases, granulated nuclei at interphase were much more prominent with polyclonal pHH3 compared to the rabbit monoclonal pHH3, making it more difficult to intrepret.[5] Rabbit polyclonal antibodies have been shown to have potential staining inconsistencies due to batch-to-batch variation and in most cases, monoclonal antibodies are generally preferred.[5] This led us to investigate the use of the rabbit monoclonal pHH3 to count MFs and to compare counts with adjacent H&E sections.

Methods and Methods

Eight cases of whole tissue formalin-fixed paraffin embedded tissues were selected. Of the 8 cases, 2 cases showed poor fixation and 6 cases showed good fixation. Adjacent sections for each case were cut at 4 microns and stained with H&E. Rabbit monoclonal pHH3 [BC39] (Biocare Medical, Concord, CA) was applied to melanoma tissue sections, followed by a polymer detection system, 3,3'-diaminobenzidine for visualization and hematoxylin counterstain. Whole slides were examined at 20X with 40X confirmation of equivocal structures. The entirety of each tumor was examined and strict criteria were used for counting mitosis: only tumor cells showing chromatin condensation and finger-like projections of chromatin, were counted as mitosis (stroma and inflammatory infiltrate excluded). Apoptotic showing breakdown of karyorrhexis were excluded.

Discussion

The 6 cases with good histology/fixation had a total of 1823 mitotic counts by H&E vs. 3703 with pHH3, thus a 203% increase in MF (Figure 2 A, B). Other studies have also demonstrated doubling of mitotic counts using polyclonal pHH3, compared to standard H&E; in line with our observations.[6-8] In the 2 cases with poor histology/fixation, 50 mitotic figures were observed by H&E vs. 265 with pHH3, representing a 530% increase in MF (Figure 3A, B). Mitotic cell counting with pHH3 was much more rapid than H&E mitotic cell counting (approximately 50% faster). We hypothesize that the overall discordance between pHH3 MFs compared to the H&Es can be explained by the high contrast and sensitivity of immunohistochemical staining for MF. Apoptotic cells and pyknotic nuclei remained unstained with pHH3, however it was noted that a few mitotic nuclei remain unstained by pHH3.
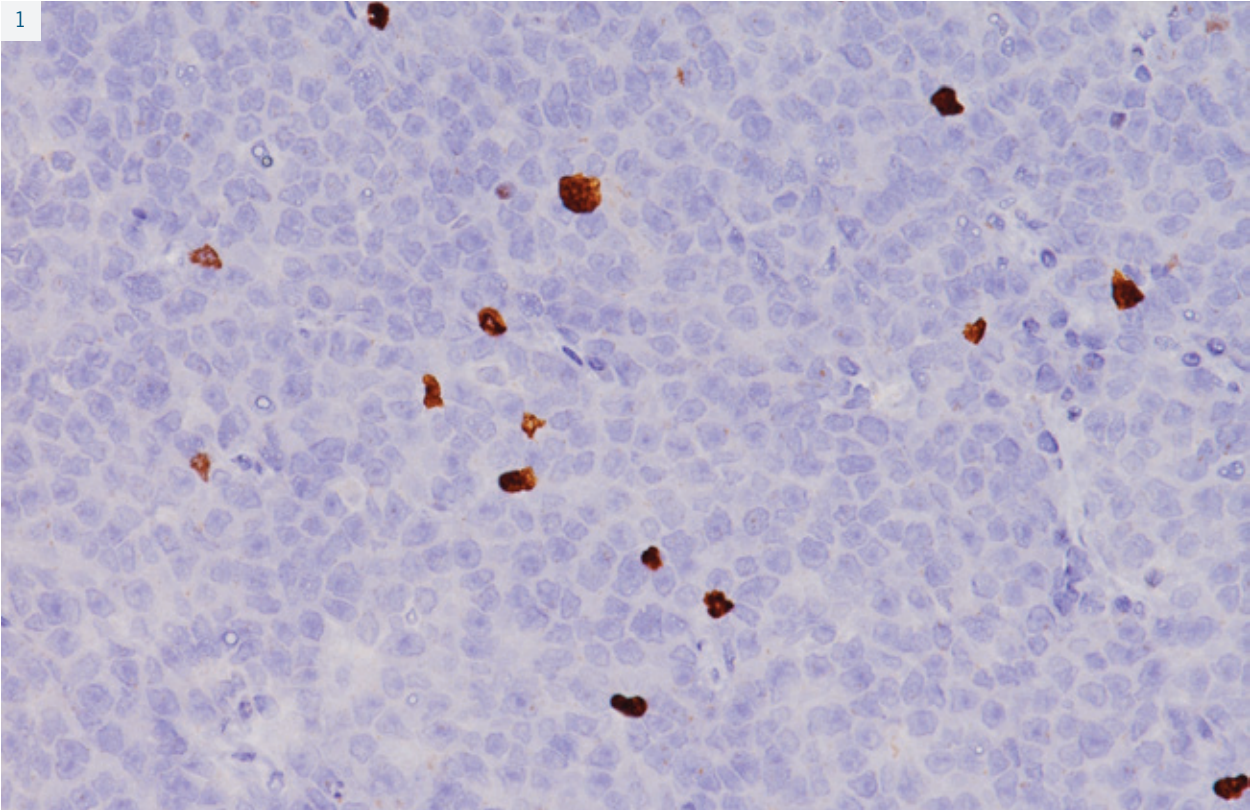
Figure 1

Figure 1: Rabbit monoclonal pHH3 in melanoma
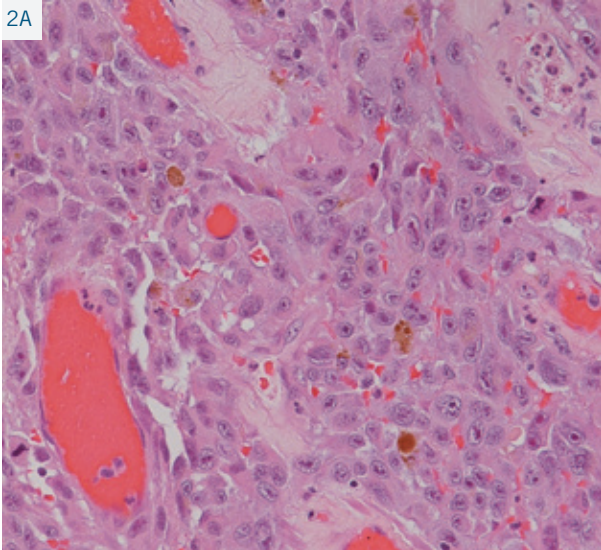
Figure 2

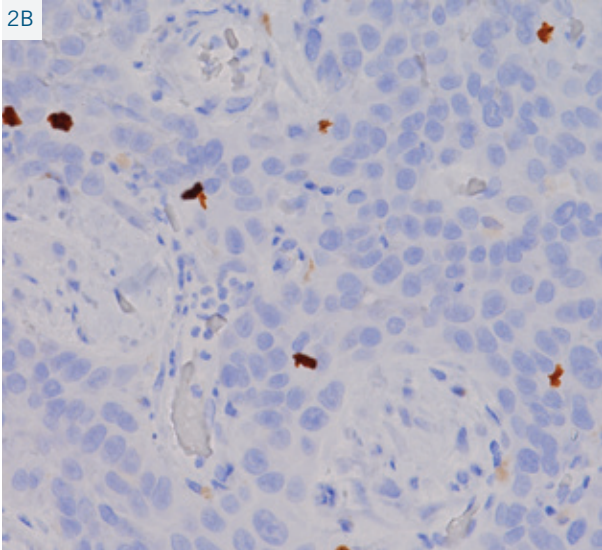Figure 2A: Melanoma, H&E Good Fixation

Figure 2B: Melanoma, pHH3 Good Fixation

Figure 3

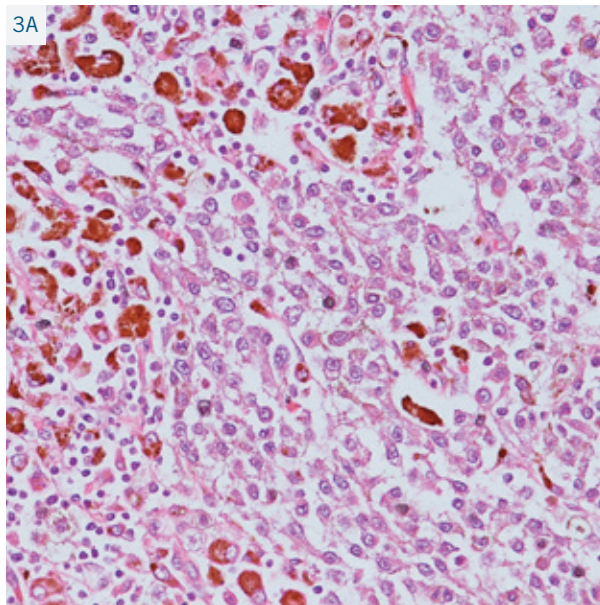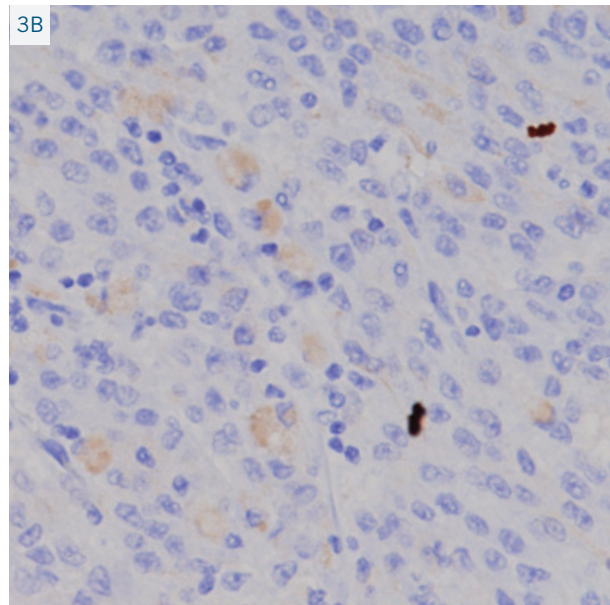Figure 3A: Melanoma, H&E Poor Fixation

Figure 3B: Melanoma, pHH3 Good Fixation

Table 1

Case	Histology Quality	Mitotic Count	pHH3
1	Good	104	214
2	Good	250	775
3	Good	972	1343
4	Good	122	512
5	Poor fixation	24	180
6	Good	118	515
7	Poor fixation	26	185
8	Good	257	344

The pHH3 antibody produced high contrast staining for MFs was observed in all 8 cases of melanoma. Manual counting MFs in tumor cells were easily calculated with a light hematoxylin stain. (Figure 1)

Conclusion

In this study, we demonstrated clear advantages of using pHH3 for obtaining mitotic count over the traditional H&E method. MFs were more easily identified following immunohistochemical staining with pHH3; and it appears to be more accurate and reproducible, especially in cases of poorly-fixed tissues. Moreover, because MFs are more easily distinguished with pHH3 staining, mitotic count analysis is significantly faster when using pHH3 over traditional H&E.

References

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