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Automated Quantification Of Proliferation With Automated Hot-Spot Selection In Phosphohistone H3/Mart1 Dual-Stained Melanomas

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Introduction/ Background

Staging of melanoma includes quantification of a proliferation index, i.e., presumed melanocytic mitoses of H&E stains are counted manually in hot spots. Yet, its reproducibility and prognostic impact increases by immunohistochemical dual staining for phosphohistone H3 (PHH3) and MART1, which also may enable fully automated quantification by image analysis.

Aims

To ensure manageable workloads and repeatable measurements in modern pathology, the study aimed to present an automated quantification of proliferation with automated hot-spot selection in PHH3/MART1- stained melanomas.

Methods

Formalin-fixed, paraffin-embedded tissue from 153 consecutive stage I/II melanoma patients was immunohistochemically dual-stained for PHH3 and MART1, and whole slide images were captured. An algorithm that automatically detects the number of PHH3/MART1-positive cells was developed using commercial software. In preprocessing, the intensity band of the HSI color model and color deconvolution including a standard deviation filter highlighted PHH3 positivity. Thresholding functions classified the image into brown PHH3, red MART1, and blue hematoxylin. Finally, postprocessing algorithms pinpointed PHH3/MART1-positive cells based on size, MART1 surrounding, color intensity, and nuclear irregularity. Based on the labels of image analysis, a hot spot was automatically selected by a processing step where circles that detect PHH3/MART1-positive cells produce a heat map according to their cluster. The number of PHH3/MART1-positive cells was counted both automatically and manually in the global tumor area and in a manually and automatically selected hot spot, i.e., a fixed 1-mm^2 square.

Results

The mean difference between manual and automated global counts was 2.9 cells/mm^2 (P = 0.0071) and 0.23 cells per hot spot (P = 0.96) for automated counts in manually and automatically selected hot spots. In 77% of cases, manual and automated hot spots overlapped. Fully manual hot-spot counts yielded the highest prognostic performance with an adjusted hazard ratio of 5.5 (95% CI, 1.3–24, P = 0.024) as opposed to 1.3 (95% CI, 0.61–2.9, P = 0.47) for automated counts with automated hot spots. In **conclusion,** the automated index and automated hot-spot selection were highly correlated to their manual counterpart, but altogether their prognostic impact was noticeably reduced. Because correct recognition of only one PHH3/MART1-positive cell seems important, extremely high sensitivity and specificity of the algorithm is required for prognostic purposes. The automated analysis may thus still aid and improve the pathologists' detection of mitoses in melanoma and possibly be useful in other malignancies and future research studies.