Laboratory Methods Supplement

Evaluating markers of epithelial-mesenchymal transition to identify cancer patients at risk for metastatic disease

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Algorithm development

After annotation algorithm (Composer) training, we developed two scoring algorithms ("solutions") per marker. All algorithms were developed using Tissue Studio. Different Composers were used to identify epithelial cell regions but identical settings were used to determine relative DAB staining intensity. The Composer_MarkerArea Solution was used to detect E-cadherin staining. This algorithm gave average intensity readings for each core on a continuous scale of 0-3 and included both membrane and cytoplasmic E-cadherin staining. We used the Composer_Nuclei(Positive_vs_Negative) Solution to detect nuclear Snail expression. Snail was measured as core percent positive nuclei on a continuous scale of 0-100.

Validation of automated analysis

To evaluate the reliability of computer annotations, one of us (ELB) used Aperio ImageScope (version 11.2; Leica Biosystem, Buffalo Grove, IL) to manually annotate the same 65 cores per marker that were used to optimize Tissue Studio solutions. He remained blind to patient and tumor characteristics while annotating. The manually-annotated cores—considered the gold standard for digital separation of tissue types—were then analyzed using appropriate Aperio

scoring algorithms (Membrane v9 algorithm for E-cadherin, Nuclear v9 algorithm for Snail). Automated scores obtained via manual and automated annotation produced Pearson correlations of 0.91 for E-cadherin and 0.94 for Snail. Having verified the accuracy of the annotation algorithms for the three markers, all 12 TMAs stained for E-cadherin and Snail were analyzed (24 slides in total).

After observing unexpected results when comparing mean Snail expression in tumor tissue to non-neoplastic tissue, we trained two additional Snail scoring algorithms on a different TMA than the one used to develop the original scoring algorithms: a second nuclear algorithm and a whole-cell algorithm that scored Snail expression in any part of a cell. These additional Snail scoring algorithms measured expression on a continuous percent positive scale and were applied to all 12 TMAs. The results of the two additional Snail algorithms were qualitatively similar to those from the original nuclear algorithm (see Online Resource 3, Table S1). Thus, for analysis we used only Snail expression values based on the original algorithm.

Future use of E-cadherin as a clinical marker

Most prior studies that measured continuous E-cadherin data did so on the percent positive scale. We encourage future investigators to adopt our use of the average intensity scale for E-cadherin, for two reasons. First, the average intensity scale imposes fewer assumptions on the data than the percent-positive and H-score scales. Second, at the level of the individual cell, the percent-positive and H-score scales assign coarser expression measurements than average intensity.

Altogether, these arguments suggest that the average intensity scale provides the richest, most-informative continuous scale on which to measure E-cadherin.

On the average intensity scale, we found that Snail had much lower expression than E-cadherin, with a high proportion of cores having expression values below the threshold for background staining (see Figure S1 in the Results Supplement for representative examples). Therefore, we suggest that the percent positive scale is appropriate for EMT inducers and mesenchymal markers, and would reserve the average intensity scale for epithelial markers.

Clinical implementation of an E-cadherin assay based on a continuous average intensity scale would require automated analysis of IHC slide images. Standardized controls with known E-cadherin staining intensities would have to be included with each run to ensure proper staining and analysis calibration. However, it would be possible to establish such an assay since whole-slide imaging for diagnostic purposes is widely used for the evaluation of estrogen receptor, progesterone receptor and HER2/Neu IHC stains. The College of American Pathologists recently released guidelines for validating new digital analysis assays for diagnostic use (1).

REFERENCE

1. Pantanowitz L, Sinard JH, Henricks WH, Fatheree LA, Carter AB, Contis L, et al. Validating whole slide imaging for diagnostic purposes in pathology: guideline from the College of American Pathologists Pathology and Laboratory Quality Center. Archives of pathology & laboratory medicine. 2013;137:1710-22.