Molecular pathology selected abstracts

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Virtual staining of tissue slides to conserve precious diagnostic samples

January 2020—Precise classification of neoplasms improves risk stratification and the ability to apply targeted treatment options, enhancing patient care. These granular diagnostic classifications increasingly rely on molecular findings that go beyond what the microscope shows the pathologist. But despite the power and promise of molecular pathology, much of a molecular pathologist's time is spent maximizing the quantity, purity, and integrity of the precious specimen undergoing expensive, laborious molecular testing. Premolecular traditional histologic evaluation of tissue or cells, or both, which is integral to selecting the correct molecular test, runs the risk of exhausting the minimal specimen before the molecular workup begins. Designing even more sensitive molecular tests that require smaller and smaller amounts of input cells or tissue is one approach to maximizing the utilization of precious pathology samples. Another approach is to reduce the premolecular consumption of specimen, leaving a greater amount for molecular analyses. Toward that end, the authors demonstrated that the autofluorescence of unstained tissue holds enough spectral information for a deep neural network to be able to virtually "color in" the tissue as if it were stained with H&E or trichrome, the latter being a more specialized stain often used to assess fibrosis. This virtual staining method, without the use of histological stains, was indistinguishable from the traditional histochemical staining methods, as assessed by a group of pathologists who compared the unstained virtual slides with histochemically stained scanned slides. The pathologists used multiple tissue types and the criteria of nuclear, cytoplasmic, and extracellular detail, and diagnosis rendered. Furthermore, the virtual staining method required only a standard fluorescent microscope and filter set for preparation, compared to histochemically staining slides, which required hazardous chemicals and a staining bench. The only modification made to the fluorescent microscope was to motorize the microscope stage. H&E and trichrome staining are consumptive steps in the premolecular evaluation of tissue biopsies, and these stained slides are no longer suitable for molecular testing as a result of being exposed to staining chemicals. Substituting a nonconsumptive virtual imaging method would permit an increased amount of precious tissue to be used for definitive molecular testing. The standard for molecular pathology practice is to sacrifice one slide for H&E staining, on which the pathologist marks the tumor-rich areas. These markings are used as a guide for scraping tissue from additional unstained slides made from adjacent sections of the paraffin-embedded tissue. This standard practice assumes, sometimes falsely, that the tissue topography does not significantly vary between the H&E slide and the unstained slides made from adjacent sections. In comparison, virtual staining allows the pathologist to evaluate and mark each unstained slide as if it had been stained with H&E, maximizing sample tumor purity and the efficiency of tissue utilization. Furthermore, if adapted to such cytology specimens as thyroid and pancreatic smears, virtual staining will allow those smears to be evaluated by a pathologist while preserving the slides for molecular evaluation.

Rivenson Y, Wang H, Wei Z, et al. Virtual histological staining of unlabelled tissue-autofluorescence images via deep learning. *Nat Biomed Eng*. 2019;3:466–477.

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Evaluating proteins simultaneously on a tissue slide to predict response to immunotherapy in melanoma

The immune system not only fights off foreign microbial invaders but also fights internal threats, such as neoplastic

processes, before they become clinically detectable cancers. Given the side effect profiles, financial costs, and opportunity costs of modern immunotherapies, it is highly desirable to have validated therapeutic and prognostic markers to guide each patient's treatment. However, just as the Mendelian principle of one gene leading to one phenotype turned out to be the exception and not the rule, finding single protein markers to predict therapeutic response is proving elusive. Therefore, there is a growing need to simultaneously evaluate multiple immune protein markers to more effectively predict optimal cancer treatments. The traditional method for studying the complex interplay of tumor and immune cells, or the tumor microenvironment, has been to evaluate one marker per section cut from formalin-fixed, paraffin-embedded (FFPE) tumor tissue. Because a single histologic section is 5 mm thick, and a typical nucleated cell is 10 to 20 mm in diameter, it is possible to query two to four protein markers in a single cell before the cell is exhausted. Consequently, the practical application of a multi-protein prognostic or theranostic signature is impossible using this traditional approach. To address this problem, the authors used a new technique to simultaneously perform 44 antibody stains to examine the expression of relevant immunoregulatory proteins in macrophages, leukocytes, and melanocytes in a cohort of 60 patients with biopsy-proven melanoma who later received PD1 and PD-L1 immune-checkpoint inhibitor therapy. All 44 protein markers were measured quantitatively in a single FFPE section. The investigators first used standard fluorescent IHC to define macrophage, leukocyte, and melanocyte cells using traditional CD68, CD45, and S100 plus HMB45 antibodies, respectively. Next, they incubated those same sections with a cocktail of 44 unique oligonucleotideconjugated antibodies. After specific antibody binding, any unbound oligonucleotide-conjugated antibody was washed away. The oligonucleotides, which served as barcodes for their respective antibodies, were then released via exposure to ultraviolet light. The oligos for each of the three cell types were collected separately via microcapillary tube inspiration for quantitative analysis. Using this novel methodology, the investigators evaluated the expression of immune protein markers in each immune cell type for their predictive value in analyzing progression-free survival and overall survival. PD-L1 expression in macrophages, which is traditionally thought to dampen immune hostility toward the expressing cell, was the best predictor of overall survival (P=.0032) and progression-free survival (P=.0072). Surprisingly, PD-L1 expression in lymphocytes and tumor cells did not have any statistically significant predictive value. These results corroborate the findings of previous studies showing that targeting PD1/PD-L1 with specific inhibitor drugs can be effective, regardless of PD-L1 expression on tumor cells. Spatially resolving the expression of molecular markers is critical to assessing the interplay of specific immune cells with tumor cells and better understanding the tumor microenvironment.

Toki MI, Merritt CR, Wong PF, et al. High-plex predictive marker discovery for melanoma immunotherapy-treated patients using digital spatial profiling. *Clin Cancer Res*. 2019;25:5503–5512.

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