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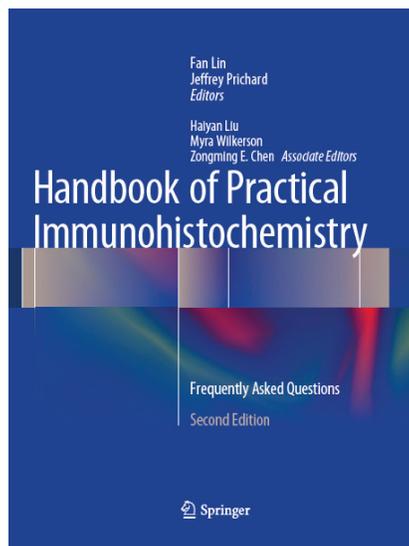
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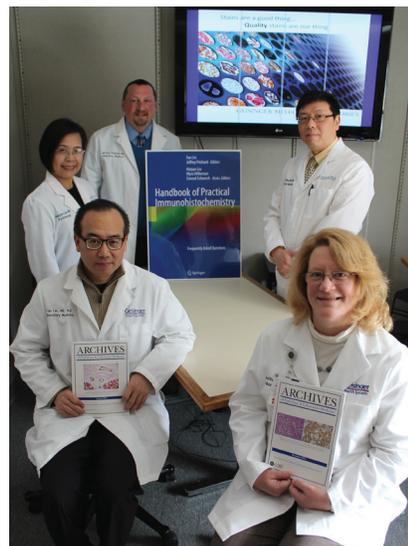
Handbook of Practical Immunohistochemistry – Second Edition

We are pleased to announce that Springer will be publishing the second edition of our *Handbook of Practical Immunohistochemistry – Frequently Asked Questions* in March. The new book will be available for your review at the USCAP meeting in Boston. Here are some significant revisions and improvements over the first edition:

1. Four new chapters and extensive additions to Chapter 2, now entitled "Standardization of Diagnostic Immunohistochemistry."
2. Over 100 new questions and answers.
3. More refined working antibody panels and more than 50 new diagnostic and predictive biomarkers.
4. More than 700 high-quality color pictures from Geisinger Medical Laboratories (GML) IHC slides.
5. More GML data. The reproducibility of antibodies reported in the literature is sometimes in question; to improve the reproducibility, we have undertaken the daunting task of testing the antibodies listed in Chapter 4 using more than 7,000 TMA and 1,500 routine slides from GML.
6. More current references.
7. A better index.



The second edition of our *Handbook of Practical Immunohistochemistry - Frequently Asked Questions* published March 2015.



Editors of the second edition of our *Handbook of Practical Immunohistochemistry - Frequently Asked Question*. Clockwise from left: Fan Lin, MD, PhD; Haiyan Liu, MD; Jeffrey Prichard, DO; Zongming E. Chen, MD, PhD; Myra Wilkerson, MD.

ALK IHC for Non-Small-Cell Lung Carcinoma

The IHC Lab has recently validated ALK immunohistochemical staining (ALK IHC) using Ventana's D5F3 clone for detection of ALK gene rearrangement in non-small-cell lung carcinoma (NSCLC). The incidence of ALK-positive NSCLC is approximately 3-4%. Because these tumors are strikingly responsive to ALK tyrosine kinase inhibitor (ALK TKI) treatments, such as crizotinib, the current practice is to screen all NSCLCs for the gene rearrangement. While FISH remains the gold standard for selecting patients for ALK TKI therapy, the 2013 CAP guideline for molecular testing of lung cancer patients¹ accepts the use of IHC as a screening methodology to select specimens for ALK FISH testing. Recent studies² also confirm high concordance between ALK IHC and ALK FISH and between evaluators. The interpretation of ALK IHC results is binary. A positive stain should show a diffuse and strong granular cytoplasmic signal in tumor cells (Figure 1), while no discernable signal is present in a negative case. For all IHC-positive or equivocal cases, ALK FISH testing is still required to confirm the presence or absence of ALK gene rearrangement. The ALK IHC test can be performed on both formalin-fixed, paraffin embedded (FFPE) tissues and cytology preparations (cell blocks). As a screening test, ALK IHC offers a cost-effective way to select patients for ALK TKI therapy by reducing unnecessary FISH tests.

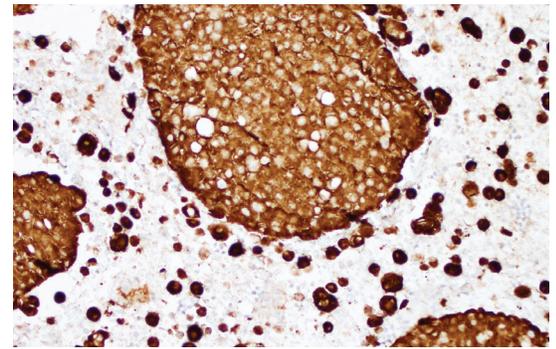


Figure 1. Positive ALK IHC stain in NSCLC (stain performed on a cell block from an FNA sample); 20X

References:

1. Lindeman NI et al. *Arch Pathol Lab Med.* 2013;137(6):828-860.
2. Wynes MW et al. *J Thorac Oncol.* 2014;9(5):631-638.

Adipophilin

Contributed by Tammie Ferringer, MD

Adipophilin is a lipid droplet-associated protein. Expression is noted in lactating mammary glands, zona fasciculata of the adrenal gland, Sertoli cells, and hepatocytes in alcoholic steatosis.^{1,2} The intracytoplasmic lipid of sebaceous tumors is highlighted with the antibody to this protein.

Sebaceous carcinoma, particularly of the eyelid, can be difficult to differentiate from squamous cell carcinoma and basal cell carcinoma. The presence of lipid by fat stains, such as Oil Red O and Sudan IV, would support sebaceous carcinoma, but this requires fresh or frozen tissue that is not typically available. Ansai et al. found that adipophilin expression supports the diagnosis of sebaceous carcinoma;³ however, the pattern of expression is important. There is membranous vesicular expression of adipophilin around the lipid vacuoles in sebaceous tumors (Figure 1), while basal cell and squamous cell carcinomas are negative or, if any expression is present, it is in a sparse granular pattern^{4,5} (Figure 2). This granular staining is likely nonspecific uptake by keratohyalin granules and the cytoplasm of macrophages.⁴

When considering the differential of clear cell neoplasms, the vesicular pattern of sebaceous tumors contrasts with the non-specific, scant granular pattern in trichilemmomas, hidradenomas, and balloon cell nevi.⁴ However, metastatic renal cell carcinoma and xanthomatous lesions, including xanthelasma and xanthogranuloma, show adipophilin reactivity in a membranous vesicular pattern similar to that in sebaceous neoplasms.⁴

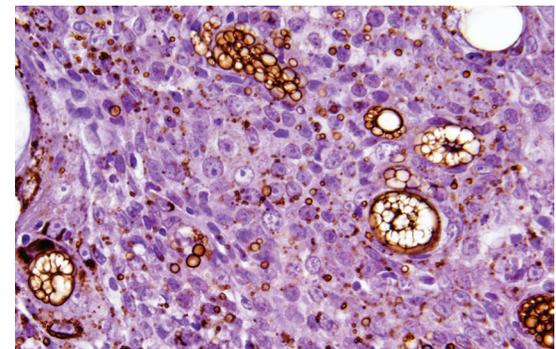


Figure 1. Membranous vesicular staining of adipophilin in sebaceous carcinoma

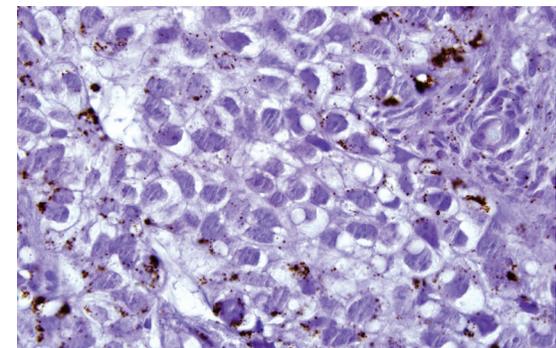


Figure 2. Sparse, non-specific granular staining of adipophilin in clear cell basal cell carcinoma

References:

1. Muthusamy K et al. *J Clin Pathol.* 2006;59(11):1166-170.
2. Heid HW et al. *Cell Tissue Res.* 1998;294(2):309-321.
3. Ansai et al. *Am J Dermatopathol.* 2011;33(6):579-587
4. Ostler DA et al. *Mod Pathol.* 2010;23(4):567-573.
5. Milman T et al. *Ophthalmology.* 2014;121(4):964-971.

Comparing Automated IHC Staining Platforms

Automation in immunohistochemistry (IHC) arose from the sharp increase in demand for stains following the discovery of heat-induced epitope retrieval (HIER) in the early 1990s. Since that time, many vendors have released platforms to manage the many steps of IHC, freeing up technologist time to perform the cutting and embedding steps that lack reasonable options for automation.

There are many factors to consider when deciding on the appropriate automated staining platform. The best way to approach this decision is to start with an understanding of your lab's needs and the proposed use. Size matters, and there must be adequate space available for the equipment and associated reagents. The capacity, speed and flexibility of batches for continuous processing should match the needs for volumes and turnaround time. The platform should provide flexibility and openness of reagent choices and protocol options to meet the number and difficulty of optimizations you will be performing. The value of automated heating to perform onboard retrieval protocols and DNA target denaturation should fit with planned testing needs. Systems vary in their ability to produce multiplexed, dual-color staining. There may or may not be value from reagent chilling capabilities, depending on the stability of chromogen substrates to be used. How a platform deals with hazardous waste can affect waste disposal costs. The software user interface should be easily understood by your staff and provide useful functionality, including operator alerts for suboptimal heating or insufficient or incorrect reagents, workload reports, stain utilization, lot-to-lot validations and reagent inventory management. Higher-volume IHC laboratories can benefit from the additional automation acquired through orders interfacing and barcode tracking with the staining platform, and, of course, the time and cost saving of the automation through better utilization of limited staff for other manual tasks should be worth the overall purchase price and operating costs of the testing system.

If an automated IHC platform is chosen correctly to match the demands of testing, automation can provide the necessary process improvement and cost savings needed in the modern practice of pathology. For a full review of current automated IHC platforms and feature comparisons, please see our review article in the December 2014 issue of *Archives of Pathology and Laboratory Medicine*.

References: Prichard JW. Arch Pathol Lab Med. 2014;138(12):1578-1582.

GEISINGER IHC

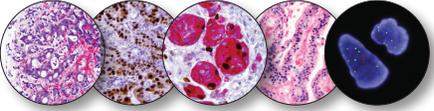
SAVE THE DATE

Friday-Sunday, September 16-18, 2016
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Program Director: **Fan Lin, MD, PhD**



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GEISINGER IHC NEWS

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