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Editor:

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Impact of Tissue Decalcification on Immunohistochemical Detection of Frequently Used Markers

Application of an immunohistochemical (IHC) assay on decalcified tissues is frequently performed in an anatomic pathology laboratory. It has been documented that tissue decalcification may have a negative impact on an assay for certain antigens. In this study, Geisinger IHC lab investigated the impact of decalcification on the detection of commonly used biomarkers using cultured cancer cell lines.

Multiple cancer cell lines (more than 20) were cultured for this study. Cell blocks were constructed from each cell line. A set of commonly used IHC markers (more than 70) was tested on selected cell lines. Cell pellets containing a mixture of the cell lines were first fixed in 10% neutral buffered formalin for 8 hours and then decalcified in Decalcifier B (Fisher Healthcare, item #23245683) for the following durations: 0 minutes (no decalcification), 30 minutes, 60 minutes, 3 hours, 6 hours, 1 day, 3 days and 1 week. Multiple tissue microarray (TMA) blocks containing these tissue/cell line cores with different decalcification times were constructed. The select markers were applied to the TMA sections using the Ventana Ultra staining platform. The staining intensity and the percentage of cells stained were recorded.

For breast carcinoma markers:

Tissue decalcification has 1) a significant negative impact on HER2 detection and on Ki67 and p53 detection after 60 minutes and 30 minutes of decalcification, respectively; 2) no negative impact on ER detection following 60 minutes of decalcification and minimal impact following 1 week's decalcification; 3) no negative impact on the detection of PR, GATA3, GCDFP15, CK7, TFF1, and TFF3 following 1 week's decalcification.

For germ cell tumor markers:

Tissue decalcification has 1) a significant negative impact on OCT4 and Nanog detection; on SOX2 detection; on SALL4, PLAP and CD30 detection; and on D2-40 detection after 30 minutes, 60 minutes, 3 hours, and 6 hours of decalcification, respectively; 2) a limited impact on the detection of glypican-3, beta-HCG, and CD10 following 1 week's decalcification.

For melanoma markers:

Tissue decalcification has a significant negative impact on SOX 10 detection and on S100, S100A6, HMB45, Mart-1, and MiTF detection after 60 minutes and 3 hours of decalcification, respectively.

For sarcoma markers:

Tissue decalcification has 1) a significant negative impact on ERG detection; on myogenin, MyoD1, NKX2.2, and Fli-1 detection; and on SMA detection after 30 minutes, 60 minutes, and 3 hours of decalcification, respectively; 2) has a limited impact on the detection of desmin and CD99 following 1 week's decalcification.

For lymphoid tumor markers:

Tissue decalcification has a significant negative impact on 79a, Tdt, and BCL2 detection; on CD2, CD5, BCL6, and EBV in situ hybridization detection; on CD3, CD10, and PAX5 detection; and on CD20 and LCA detection after 30 minutes, 60 minutes, 3 hours, and 6 hours of decalcification, respectively.

For select markers for carcinoma of unknown origin:

Tissue decalcification has little impact on the detection of CDX2, PR, chromogranin, calcitonin, BerEP4, S100P, GCDFP15, p16, P504S, vimentin, CK7, CK20, CK5/6, and CD56 after 1 week's decalcification. The impact of decalcification on other tested IHC markers is summarized in the table below.

Time in Decalcification	Significant negative impact on tested IHC markers
Any time in decalcification	HPV in situ hybridization
After 60 minutes	SATB2, PAX8, ER, maspin, IMP3, p40, p63
After 3 hours	TTF1
After 1 day	Synaptophysin, MUC1, MUC2, beta-catenin
After 3 days	CEA

These data demonstrate that tissue decalcification has various impacts on antigen detection. These data can serve as a reference for these particular markers. Caution should be taken when performing an IHC assay on decalcified tissues.

References:

Lin F, Shi J, Chen Z, Liu H. Impact of tissue decalcification on immunohistochemical detection of select markers for carcinoma of unknown origin [USCAP abstract 2081]. *Mod Pathol*. 2015; 28(S2):518A.

Jianhui Shi, Vivian Xu, Haiyan Liu, Fan Lin. Impact of tissue decalcification on immunohistochemical detection of breast carcinoma markers [USCAP abstract 251]. *Mod Pathol*. 2015; 28(S2):66A

Liu H, Shi J, Xu V, Lin F. Impact of tissue decalcification on immunohistochemical detection of select sarcoma markers [USCAP abstract 71]. *Mod Pathol*. 2015; 28(S2):21A.

Summary of Abstracts from the 2015 USCAP Meeting (Part 1 of 2)

[1765] Tissue Transglutaminase II is a Novel Biomarker for Pancreatic Ductal Adenocarcinoma on Core Needle Biopsy and Resection Specimens. *Todd DeJulio, et al.*

Tissue transglutaminase II (TGM-2) IHC showed that 80% (20/25) of pancreatic ductal carcinomas on core needle biopsy and 78% (54/69) on resected specimens showed diffuse and strong positive staining for TGM-2. All six of the benign pancreatic core needle biopsy cases and all adjacent normal pancreatic parenchyma in the resected specimens showed negative staining for TGM-2. Capillary endothelial cells and desmoplastic stroma adjacent to invasive carcinoma in the resected specimens displayed intense TGM-2 staining. These results indicate that TGM-2 is a novel biomarker for pancreatic ductal adenocarcinoma that is sensitive and specific and is useful in supporting the diagnosis on core needle biopsies. Overexpression of TGM-2 in cancer-adjacent endothelial cells and desmoplastic stroma implies its role in tumor-stromal interaction and angiogenesis.

[1766] Expression of TTMP – A Novel Growth Inhibition Gene Is Lost in Pancreatic Neuroendocrine Tumor. *Xianzhong Ding, et al.*

Immunostains show a strong TPA-induced transmembrane protein (TTMP) expression (2+) in normal pancreatic islet cells with an intracytoplasmic granular staining pattern. In contrast, there is a complete loss of TTMP expression in all of the well differentiated pancreatic neuroendocrine tumors tested (100%; 19/19). Pancreatic acinar and ductal cells are positive for TTMP but with only very weak cytoplasmic staining (0 to 1+). In vitro study shows TTMP overexpression significantly inhibits human cell proliferation ($p < 0.01$). Expression of TTMP is completely lost in well differentiated pancreatic neuroendocrine tumors (PNETs), while TTMP overexpression blocks cell proliferation. These results suggest that TTMP might be involved in pancreatic neuroendocrine tumorigenesis and progression. TTMP could be a useful marker in distinguishing PNETs from normal pancreatic islets.

[890] PD-L1 Expression by Immunohistochemistry in Clear Cell Renal Cell Carcinoma Is Associated With High Tumor Stage and Nuclear Grade. Steven M Goodman, et al.

In a patient cohort of 354 clear cell renal cell carcinomas (CCRCCs), programmed death ligand 1 (PD-L1) expression was shown to be associated with high tumor stage and high Fuhrman nuclear grade. A total of 55% of tumors with PD-L1 expression were stage IV, and 83% were high nuclear grade. In contrast to previously published studies, our series did not show correlation with overall survival. Our study further demonstrates the high grade and high stage nature of PD-L1-expressing tumors in which PD-L1 expression may be considered a potential target for personalized therapy.

[904] PAX8 and GATA3 Immunophenotypes in Upper Tract Urothelial Carcinoma (UC): Emphasis on Variation by Anatomic Site and in Histologic Variants. Holly Harper, et al.

GATA3 shows strong and diffuse staining in the majority of upper tract UCs, including both invasive and non-invasive components, but may be more focal in histologic variants. PAX8 showed immunoreactivity in 42% of cases and was more common in the renal pelvis (50%). Although PAX8 expression was often lost in variant patterns of UC, it was maintained in sarcomatoid UCs, underscoring the need for caution in the distinction from sarcomatoid renal cell carcinoma.

[954] Immunohistochemical Evaluation of NKX3.1 Expression in 1864 Human Tumors. Haiyan Liu and Fan Lin.

The staining results showed that 100% of prostatic adenocarcinomas (ADCs) expressed NKX3.1, with diffuse nuclear staining in the majority of cases. The same pattern was seen in benign prostatic tissues, whereas none of the benign seminal vesicle tissues was positive. Eleven percent of invasive lobular carcinomas (CAs) and 33% of invasive ductal CAs of the breast also expressed NKX3.1. All other tumors, except a pulmonary squamous cell carcinoma (SqCC), were negative for NKX3.1, including germ cell tumors of the testis. These data demonstrate that NKX3.1 is a highly sensitive and relatively specific prostate immunomarker, with the exception of mammary CAs. NKX3.1 should be included in the panel of immunomarkers for the identification of prostate origin when working on metastatic tumors of unknown primary.

[534] Immunohistochemical Expression of NKX2.2 in 1434 Human Tumors. Haiyan Liu and Fan Lin.

NKX2.2 was tested in 1434 tumor cases, including 198 neuroendocrine tumors (NETs). Of the 198 NETs, colorectal NETs showed the highest expression of NKX2.2 (72%); NETs of the pancreas, stomach and small intestine expressed NKX2.2 in slightly over 50% of cases, while the majority of pulmonary neuroendocrine carcinomas and cutaneous NETs lacked NKX2.2 expression. Other non-NETs, except one endometrial adenocarcinoma (ADC) and one lung ADC, were negative for NKX2.2. Our data suggest that NKX2.2 is a highly specific

and relatively sensitive immunomarker for NETs, especially for gastrointestinal (including pancreatic) primary. NKX2.2 can potentially serve as a neuroendocrine marker in daily practice.

[634] Co-Expression of MUC5AC and TFF1 Distinguishes Sessile Serrated Adenomas/Polyps from Hyperplastic Polyps. Hannah E Goynes, et al.

Normal colon samples had no expression of MUC5AC, while one had mild TFF1 staining. Hyperplastic polyps (HPs) showed patchy, weak staining with MUC5AC and variable TFF1 expression. Sessile serrated adenomas/Polyps (SSA/Ps) exhibited strong, diffuse staining for both. The differences in average expression scores of SSPs and HPs for MUC5AC, TFF1 and MUC5AC/TFF1 co-expression were statistically significant (two-tailed t-test). Strong and diffuse co-expression of MUC5AC and TFF1 is seen in SSA/Ps, consistent with previous RNA sequencing data. Further study is needed, but these findings may help distinguish SSA/Ps from HPs in diagnostic practice.

[564] NKX2.2 Expression in Well Differentiated Neuroendocrine Tumors. Chaohui Zhao, et al.

Normal pancreatic islets and scattered gastric and intestinal neuroendocrine cells demonstrated strong nuclear NKX2.2 staining. NKX2.2 was frequently positive in rectal (95%), duodenal (88%), appendiceal (79%), small intestinal (60%), and pancreatic (59%) well differentiated neuroendocrine tumors (WDNETs). In pancreatic and rectal WDNETs, staining for NKX2.2 was strongly associated with Islet 1 and PAX8 ($P < 0.0001$). Immunoreactivity for all three markers was concordant in the vast majority of pancreatic WDNETs (82.4%), including 8 cases positive, and 4 cases negative for all three markers. Lower NKX2.2 positivity (36%) was seen in gastric carcinoids, while all pulmonary carcinoids were negative. In contrast to NKX2.2, all small intestinal WDNETs were negative for Islet 1, and only 5% were positive for PAX8. CDX2 was expressed predominantly in small intestinal (84%) and appendiceal (87%) WDNETs, while TTF-1 immunoreactivity was restricted to pulmonary carcinoids. This study is the first to comprehensively examine NKX2.2 expression in a wide spectrum of WDNETs. NKX2.2 is variably expressed in digestive tract WDNETs and is negative in pulmonary carcinoids. NKX2.2 is as sensitive as Islet 1 and PAX8 for pancreatic WDNETs; however, its ability to distinguish pancreatic from gastrointestinal WDNETs is limited as compared to Islet 1 and PAX8.

Complete abstracts can be found in *Mod Pathol.* 2015;28(S2).

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100 North Academy Avenue
Danville, PA 17822-0131
1.800.695.6491

www.geisingerihc.com