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Ribonucleic acid *in situ* hybridization (RNAscope) is a more sensitive method than immunohistochemistry in detection of TTF-1 and napsin A expression in lung adenocarcinomas

TTF-1 and napsin A immunomarkers play a crucial role in differentiating lung adenocarcinoma from lung squamous cell carcinoma and identifying a primary lung adenocarcinoma when working on a tumor of unknown origin.

We investigated the diagnostic sensitivity of ribonucleic acid *in situ* hybridization (RNAscope; Advanced Cell Diagnostics; Hayward, Calif.) in the detection of expression of these biomarkers in lung adenocarcinomas and compared it to immunohistochemical techniques.

Both RNAscope and immunohistochemical assays for TTF-1 and napsin A were performed on tissue microarray sections containing 80 lung adenocarcinomas and 80 lung squamous cell carcinomas. RNAscope assay for both TTF-1 and napsin A was also performed on 220 adenocarcinomas from various organs.

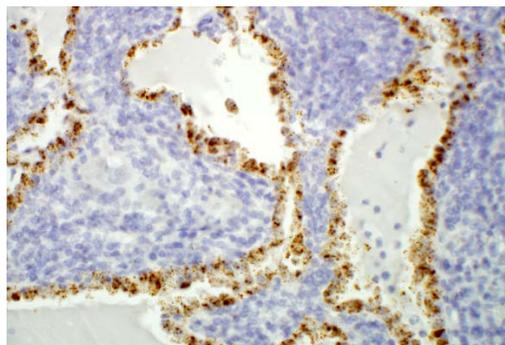


Figure 1: High-level expression of TTF-1 detected by RNAscope.

RNAscope for TTF-1 was positive in 92.5% of lung adenocarcinomas; in contrast, immunohistochemistry was positive in 82.5%. RNAscope for napsin A was positive in 90% of lung adenocarcinomas; immunohistochemistry was positive in 77.5%.

Napsin A expression was not seen in lung

squamous cell carcinomas by either method. In contrast, TTF-1 expression was seen in 3.75% (1+) and 10% (1+) of squamous cell carcinomas by immunohistochemistry and RNAscope, respectively.

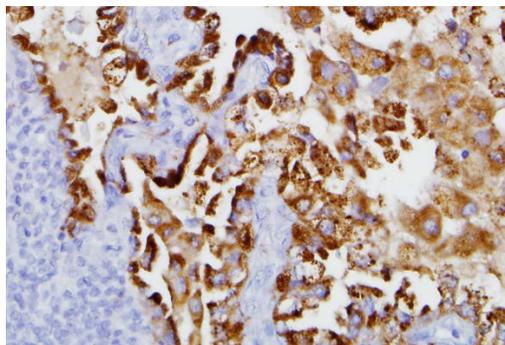


Figure 2: High-level expression of napsin A detected by RNAscope.

All non-pulmonary adenocarcinomas were negative for TTF-1 by RNAscope assay. An example of lung adenocarcinoma with a high-level expression of TTF-1 and napsin A by RNAscope is shown in Figure 1 (TTF-1) and Figure 2 (napsin A), respectively.

Preliminary data suggest that RNAscope is superior to immunohistochemistry in detecting TTF-1 and napsin A expression in primary lung adenocarcinomas. Therefore, performing an RNAscope assay may be considered for both TTF-1- and napsin A-negative cases with a clinical suspicion of lung adenocarcinoma. TTF-1 results should be interpreted with caution since a small percentage of squamous cell carcinomas can be focally positive by either assay.

The utility of RNAscope in the detection of other diagnostic IHC markers with relatively low diagnostic sensitivity, such as GCDFP-15 and uroplakin II, is currently under investigation.

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Summary of Abstracts from 2015 USCAP meeting (Part 2 of 2)

[1016] DOG-1 Immunohistochemical Staining in Testicular Biopsies Is a Reliable Tool for Objective Assessment of Infertility. *Rasha Salama, et al.*

Evaluation of infertility in testicular biopsies requires assessment of spermatogenesis and maturation of germ cells in a stepwise fashion. DOG-1 is expressed in spermatocytes and spermatids but not in Sertoli cells and spermatogonia. By using the DOG-1 stain we were able to confirm maturation arrest and determine the level at which maturation stops. This may have pathogenetic importance, as different processes cause maturation arrest at different levels. Moreover, we could objectively assess the percentage of the testis involved by hypospermatogenesis in mixed patterns. Despite its positivity in normal spermatocytes, DOG-1 is negative in spermatocytic seminomas.

[897] The Utility of STAT6 Expression in the Differential Diagnosis of SFT Vs Stromal Prostatic Neoplasms. *Gunes Guner, et al.*

A total of 31 cases (11 needle biopsies, 13 radical retropubic prostatectomy [RRP] and 7 transurethral resection of the prostate [TURP]) were retrieved from the authors' surgical pathology and consultation archives (1995–2014). They included 15 stromal tumors of unknown malignant potential (STUMPs), 11 solitary fibrous tumors (SFTs) and 4 low-grade prostatic stromal sarcomas. Sections were stained with polyclonal STAT6 antibody (Santa Cruz, S20, 1:100). Only unequivocal nuclear staining (with or without cytoplasmic staining) was considered positive. Cases with "background" nuclear staining in glandular epithelium or lymphocytes were excluded from the analysis. All 9 evaluable SFT cases demonstrated nuclear STAT6 positivity. None of 10 evaluable

STUMP cases had nuclear staining (see Table below). All of the 4 low-grade stromal sarcomas of the prostate were STAT6-negative.

STAT6 status	SFT	STUMP	Stromal sarcoma
Positive	9	0	0
Negative	0	10	4

STAT6 is a useful marker in the differential diagnosis of SFT vs stromal prostatic tumors that will complement existing morphologic and immunohistochemical criteria for the diagnosis of mesenchymal lesions of the prostate.

[592] LIN28 Was Expressed in Gastric Hepatoid Adenocarcinoma: An Immunohistochemical Study of 33 Cases With Comparison To SALL4, AFP, Glypican-3, Hepatocyte and Polyclonal-CEA. *Dengfeng Cao, et al.*

Thirty-three primary gastric hepatoid adenocarcinomas (PGHAs) were included for this study. Immunohistochemical staining was performed with antibodies to the following proteins: LIN28, SALL4, AFP, GPC3, hepatocyte and p-CEA. Only nuclear staining was considered positive for SALL4. Only a canalicular pattern was considered positive for p-CEA.

Positive staining with LIN28, SALL4, AFP, GPC3, hepatocyte and p-CEA was seen in 16/33 (48%), 27/33 (82%), 25/33 (76%), 22/33 (67%), 18/33 (55%) and 17/33 (52%) PGHAs, respectively. LIN28 and SALL4 demonstrated the following combinations: SALL4+/LIN28+ in 11/33 (33%, either AFP or GPC3+ in 11/11), SALL4+/LIN28- in 15/33 (45%, either AFP or GPC3+ in 12/15), SALL4-/LIN28+ in 6/33 (18%, either AFP or

GPC3+ in 5/6), and SALL4-/LIN28- in 1/35 (3%, AFP+ in 1/1). LIN28 was expressed in approximately half (48%) of PGHAs and was not as sensitive as other markers. Among the 6 markers, SALL4 is the most sensitive, and its diagnostic sensitivity can be further enhanced in combination with LIN28. However, the maximal diagnostic sensitivity was only achieved with a panel including LIN28, SALL4, AFP and GPC3.

[713] CD200 Is Expressed in Gastrointestinal Neuroendocrine Tumors and Correlates With Grade. Claire Murphy, et al.

A total of 113 resected neuroendocrine neoplasms (NENs) of the pancreas (47), small bowel (51), colon (4) and appendix (11) were assessed for mitotic count (MC), necrosis and immunohistochemical staining for Ki67, PHH3, and CD200. Grade (G1-3) was based on MC and Ki67, in accordance with the 2010 WHO classification. Grade based on PHH3 was determined by counting PHH3+ cells in 50 HPF and stratified using the same criteria for MC. CD200 staining was scored as positive or negative. Clinical data were obtained from chart review and from the National Death Index. Fisher’s exact test was used to examine the significance between CD200 expression and tumor grade. The mean square contingency coefficient between CD200, MC, and Ki67 was also calculated to determine the strength of the association.

93% (105/113) of the NENs showed diffuse positivity for CD200. The higher grade NENs (G2-3) were more likely to be CD200-negative ($p < 0.001$) and the strength of this association was high ($\Phi = -0.4$). This association between higher grade and CD200 negativity was regardless of grading method used (Ki67 index, MC, PHH3 count, $p < 0.001$ for each measure). Of the 3 markers, CD200 expression was most strongly associated with Ki67 index. There was also a trend towards an association between the CD200 negativity and the presence of necrosis, but this did not reach statistical significance ($p = 0.056$). At a median follow-up of 53 months, the median survival of the population had not been reached. However, 62% of the patients with CD200-negative NENs were deceased compared to 12% of patients with CD200+ NENs.

CD200 is positive in the majority of GI NENs, and negative staining is strongly associated with higher tumor grade. In this study, patients have an overall excellent prognosis after resection (evidenced by the median survival not being reached). However, of the patients with CD200-negative NENs, 62% have died, indicating that CD200 may also be helpful in evaluating prognosis.

[751] Expression of CD24, a Stem Cell Marker, in Pancreatic and Small Intestinal Neuroendocrine Tumors. Safia Salaria, et al.

Well-differentiated neuroendocrine tumors (NETs) of the gastroenteropancreatic system comprise approximately two-thirds of total NETs, and the pathogenesis of foregut NETs is distinct from that of midgut NETs. CD24 has been recognized as both a normal and a malignant stem cell biomarker; despite extensive studies, its expression in intestinal and pancreatic NETs remains unknown. In this study, the authors wanted to determine CD24 expression in pancreatic and small intestinal NETs.

They observed scattered CD24-positive cells in the duodenal and ileal crypts, most of which showed a strong subnuclear labeling pattern. The same pattern of the expression was observed in 95% of primary ileal NETs, but only in 15% of duodenal NETs ($p < 0.01$). In addition, metastatic ileal NETs retained CD24 expression. Pancreatic islets did not express CD24, and only rare cells had subnuclear labeling of CD24 in the pancreatic ducts. Unlike ileal NETs, only 5% of pancreatic NETs expressed CD24 in the subnuclear compartment ($p < 0.01$). In particular all 5 of these NETs shared unique morphologic features, including dense fibrosis, small nests/tubules and an infiltrative growth pattern.

See Table 1. Expression of CD24 in Duodenal, Midgut and Pancreatic Neuroendocrine Tumors

Immunohistochemical studies for CD24 expression might have potential clinical implications. Ninety-five percent of midgut NETs expressed CD24, whereas only 5% of pancreatic and 15% of duodenal NETs had CD24 expression. Importantly, metastatic midgut NETs retained subnuclear CD24 expression. We propose CD24 has a novel marker for identifying primary midgut neuroendocrine tumors as well as a subset of primary pancreatic neuroendocrine tumors with a unique histomorphologic pattern of disease. The distinction is important, as CD24 has potential as a small intestinal cancer stem cell marker; CD24-reactive NETs could be prospective candidates for both anti-cancer stem cell and anti-angiogenic therapeutic regimens.

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

Table 1. Expression of CD24 in Duodenal, Midgut and Pancreatic Neuroendocrine Tumors

CD 24+	Duodenal NETs (n=26)	Midgut NETs		Pancreatic NETs	
		Primary (n=43)	Liver Metastasis (n=18)	Primary (n=92)	Liver Metastasis (n=18)
	4 (15%)	41 (95%)	17 (94%)	5 (5%)	0 (0%)

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