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Distinguishing Adenocarcinoma from Squamous Cell Carcinoma in the Lung Using Multiplex IHC Stains: p63 + CK5 and TTF-1 + Napsin A

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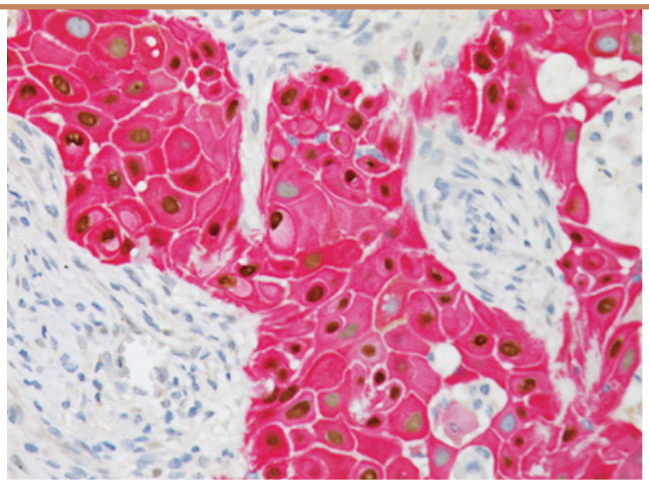

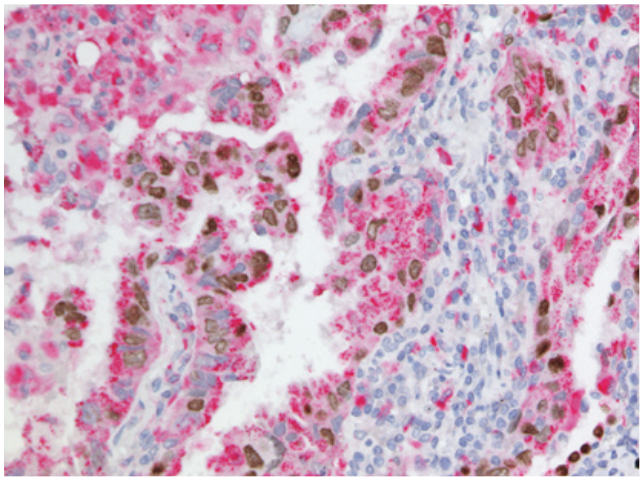

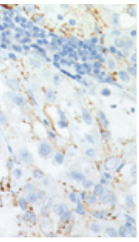

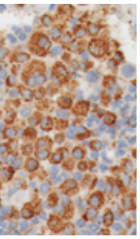

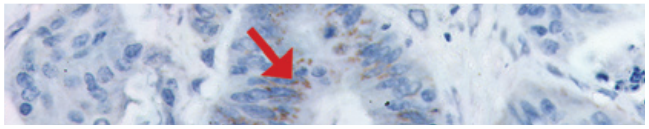

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Background

The current FDA-approved standard treatment for non-small cell lung cancer is Carboplastin/Taxol/Avastin; however, based upon survival benefit, patients with squamous cell carcinoma (SqCC) should not receive Avastin due to a 30% mortality rate by fatal hemoptysis. Further, selection of therapies such as VEGR and EGFR inhibitors may depend on the correct differentiation of SqCC vs. lung adenocarcinoma (LADC) of the lung. TTF-1, CK7 and p63 expression have been used to differentiate primary lung cancers; however, the need for a sensitive and specific panel of antibodies to differentiate lung adenocarcinoma from lung SqCC is of the utmost importance. Thrombomodulin (CD141), p63, 34betaE12 and CK5/6 have been shown to be sensitive markers for SqCC. TTF-1 and Napsin A have been shown to be specific markers for lung adenocarcinoma; however, the specificity of Napsin A for other cancer types is not completely known. We investigated the specificity and sensitivity of Napsin A on a wide spectrum of neoplastic tissues including lung cancers. Antibodies TTF-1, Napsin A, CK5, CK5/6, p63, CD141 and 34betaE12 were evaluated individually to increase sensitivity and specificity for differentiation of LADC from SqCC, and for incorporation of these antibodies into potential Multiplex IHC stain applications.

Methods

Tissue microarrays (TMA) were constructed from archival normal and neoplastic tissues and/or purchased from commercial sources. TMA slides were deparaffinized and antigen retrieval was performed. Napsin A was evaluated for specificity and sensitivity on a diverse range of neoplastic tissues and on different lung cancer subtypes (n=60). A comparison of 80 cases of lung cancer were evaluated with TTF-1, Napsin A (monoclonal/polyclonal), CD141, p63, CK5 (rabbit monoclonal [RM]) and 34betaE12. We also evaluated a well published mouse monoclonal CK5/6 [D5/16B4] in comparison to a new rabbit monoclonal CK5 for specificity and sensitivity in lung cancers (n=60). Napsin A [TMU-Ad 02] was also compared to a polyclonal Napsin A for specificity and sensitivity using commercial TMAs that included lung and other cancers. All Multiplex IHC stains were performed utilizing a primary cocktail of mouse and rabbit antibodies, followed by a cocktail of conjugated goat anti-mouse horseradish peroxidase (HRP) and conjugated anti-rabbit alkaline phosphatase (AP) (Biocare Medical). A dual color reaction was accomplished by applying DAB and Vulcan Fast Red.

Results

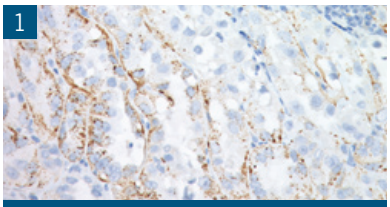
Napsin A (polyclonal/monoclonal) was positive in 43% of renal cell carcinomas including papillary, chromophobe and clear cell RCC (photo 1), 21% of thyroid cancers; 4% of ovarian cancers; 17% of cervical adenocarcinomas, and was 100% negative in all cervical squamous cell carcinomas (Table 1). Colon cancers were 100% negative for Napsin A mouse monoclonal. The Napsin A polyclonal was positive in only 8/79 cases (only tubular adenocarcinomas) (photo 2); however only grades 1-2 stained positive, and staining patterns were not characteristic of lung cancer (photo 3). Cases that were grade 3 and 4, and all metastatic colon cancers were 100% negative (data not shown). Breast, prostate, bladder, stomach, seminoma, liver, bile duct, lymphoma, leiomyosarcoma, melanoma and pancreatic cancers were all negative (Table 1). When the same tissues were stained with Napsin A (M), data was equivalent except for colon (Table 1).

In LADC, Napsin A staining was highlighted by granular cytoplasmic staining around the nuclei (Photo 3), and demonstrated equal sensitivity to TTF-1 (79%), but was slightly more specific (Table 2). There was one case of LADC that was TTF-1 (+) and Napsin A (-), and one case that was TTF-1 (-) and Napsin A (+). TTF-1 was positive in 6% of SqCC and Napsin A was negative in 100% of SqCC. The combination of TTF-1 + Napsin A demonstrated 100% specificity for LADC. We later added CK5 and 34betaE12 antibodies to our original study. The co-expression of p63 and CK5 provided 100% specificity for SqCC (Table 2). 34betaE12 had the highest sensitivity for SqCC (94%), but also stained 27% of LADC. Rabbit monoclonal CK5 was 100% specific for SqCC and 100% negative for LADC. CD141 was 70% sensitive for SqCC (Table 2). There were 2 cases of SqCC that did not express p63, CK5, 34betaE12 or CD141, but expressed both Napsin A and TTF-1. These cases were further evaluated and were classified as non-squamous cell carcinomas. There were seven cases of lung cancer (9%) that were completely negative for p63, CK5, TTF-1 and Napsin A. CK5 was determined to be slightly more sensitive for staining SqCC when compared to CK5/6 (79% vs. 75%, n=32), and both antibodies were 100% negative for LADC (n=28). In LADC, Napsin A (P) was more sensitive than mouse monoclonal Napsin A (88% vs. 84%, n=32), and both antibodies were 100% negative for SqCC (n=28). It was determined that the combination of TTF-1 (M) + Napsin A (P) and p63 (M) + CK5 (RM) (photos 4-5) were suitable for Multiplex IHC stains and performed equally well when compared to single stains (Table 2).

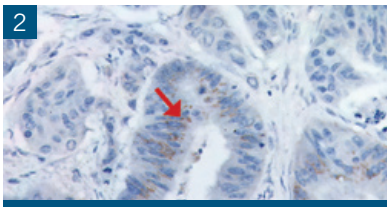
Conclusion

We have demonstrated Napsin A as a useful marker for recognition of primary pulmonary adenocarcinoma, with some limitations (mainly its expression in renal cell carcinomas). Napsin A is similar in sensitivity to TTF-1, but displays higher specificity. These results indicate that Napsin A is a promising marker for differential diagnosis of LADC and squamous cell carcinoma. The co-expression of TTF-1 + Napsin A was both sensitive and highly specific for LADC. Likewise, the co-expression of p63 + CK5 provided the highest sensitivity combined with 100% specificity for SqCC. For primary lung cancer cases, this combination of Multiplex IHC tests is 100% specific for SqCC and 79% sensitive for LADC. We also demonstrated the utility of two Multiplex IHC stains. The TTF-1 + Napsin A Multiplex IHC and the p63 + CK5 Multiplex IHC cocktails combine to simplify interpretation and provide increased specificity and sensitivity in differentiation of squamous vs. non-squamous carcinomas, a clinically important distinction in light of current therapeutic recommendations.

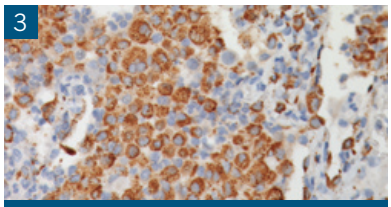
Photos 1-3 stained with Napsin A



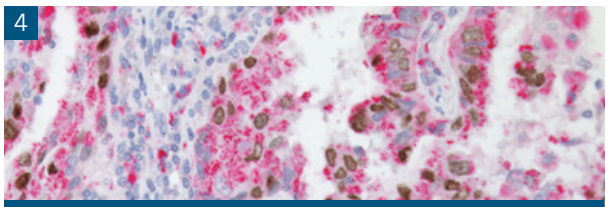
Clear cell renal cell carcinoma



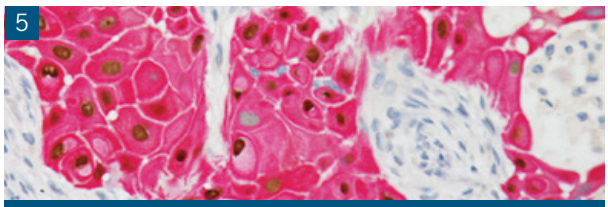
Colon adenocarcinoma



Lung adenocarcinoma



TTF-1 (DAB) + Napsin A (Fast Red) Multiplex IHC stain



p63 (DAB) + CK5 (Fast Red) Multiplex IHC stain

Table 1: Napsin A (P) - Non-lung Tumors

Multiple cancers	Antibody	Cases	Positive	% Positive	% Negative
Renal cell carcinoma	Napsin A	40	17	43%	57%
Ovarian cancer		54	2	4%	96%
Colon cancer		79	8*	10%	90%**
Thyroid cancer		28	6	21%	79%
Bladder cancer		36	0	0%	100%
Cervical cancer (Squamous)		30	0	0%	100%
Cervical cancer (Adenocarcinoma)		6	1	17%	83%
Breast cancer		41	0	0%	100%
Prostate cancer		25	0	0%	100%
Pancreatic cancer		10	0	0%	100%
Melanoma		14	0	0%	100%
Stomach cancer		10	0	0%	100%
Seminoma		10	0	0%	100%
Cholangiocarcinoma		4	0	0%	100%
Hepatocellular carcinoma		12	0	0%	100%
Leiomyosarcoma		6	0	0%	100%
Lymphoma		1	0	0%	100%

* Positive in grade 1 and 2 tubular carcinomas only

* Monoclonal Napsin A was 100% negative in all colon cancers

Table 2: Antibodies Tested for Lung Cancer

Multiple cancers	Antibody	Cases	Positive	% Positive	% Negative
Adenocarcinoma	Napsin A	33	26	79%	21%
	TTF-1	33	26	79%	21%
	TTF-1 + Napsin A	33	28	85%	15%
	CD141	33	4	12%	88%
	p63	33	2	6%	94%
	CK5	33	0	0%	100%
	34betaE12	33	9	27%	73%
SqCC	Napsin A	47	0	0%	100%
	TTF-1	47	3	6%	94%
	CD141	47	33	70%	30%
	p63	47	41	87%	13%
	CK5	47	38	81%	19%
	p63 + CK5	47	41	87%	13%
	34betaE12	47	44	94%	6%

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