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TTF-1, Napsin A, p63, TRIM29, Desmoglein-3 and CK5: An Evaluation of Sensitivity and Specificity and Correlation of Tumor Grade for Lung Adenocarcinoma versus Squamous Cell Carcinoma.

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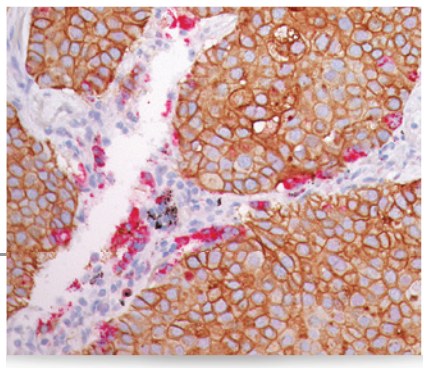
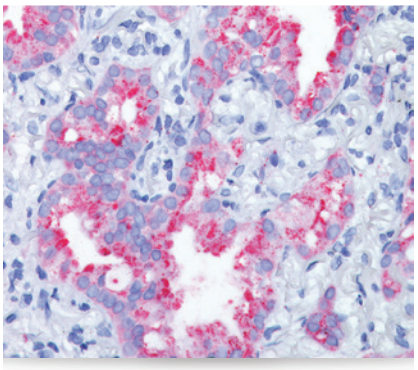
Adenocarcinoma VS. Squamous Cell Carcinoma





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Background

The current FDA-approved standard of treatment for non-small cell lung cancer (NSCLC) is Carboplastin/Taxol/Avastin; however, patients with lung squamous cell carcinoma (SqCC) should not receive Avastin due to a 30% mortality rate by fatal pulmonary hemorrhage. Antibodies TTF-1 and p63 have been used to differentiate primary lung cancers and recently CK5/6 and Napsin A have been reported; however, the need for a more sensitive and specific panel of antibodies to differentiate lung adenocarcinoma (LADC) from lung SqCC is of the utmost importance. In a pilot study, 12 antibodies were evaluated using an immunohistochemical (IHC) method (see Table 1). Based on sensitivity and specificity determined in the pilot study, six antibodies were selected for further evaluation on 210 various lung cancer cases. Observed staining patterns were correlated with tumor grade.

Design

Formalin-fixed paraffin-embedded TMA tissues for lung cancers were obtained and processed in the usual manner for IHC analysis. All sections were retrieved in a modified citrate buffer formula (DIVA, Biocare Medical) and placed in a pressure cooker at 125°C. TTF-1, Napsin A, p63, TRIM29, Desmoglein 3 and CK5 were optimized with custom diluents and were applied to 95 cases of lung SqCC and 115 cases of LADC. Detection was achieved using a micro-polymer detection system (MACH 2, Biocare Medical) and visualized with DAB and/or Fast Red. Single stains and multiplex stains were developed for IHC analysis and comparisons. For each antibody tested, cases were considered positive if 10% or more tumor cells were stained. Cases with <10% staining and no focal areas of positive staining were scored as negative. Cases that were mostly negative, but contained small areas of tumor cells in which almost all tumor cells were positive were classified as focally positive.

Results

A six antibody panel was evaluated for sensitivity and specificity on 95 cases of SqCC and 115 cases of LADC (Table 2). Positive staining with Napsin A and/or TTF-1 provided 91.3% sensitivity and 94.7% specificity for LADC ($P < 0.0001$) (Table 2). Desmoglein-3 and/or CK5 resulted in 92.6% sensitivity and 100% specificity for SqCC ($P < 0.0001$). Additionally, p63 and/or TRIM29 provided 94.7% sensitivity and 89.6% specificity for SqCC ($P < 0.0001$).

In all cases, 15/210 (7.1%) were unclassified by the six antibody panel (Table 2). Specifically, 10/115 (8.7%) LADC and 5/95 (5.3%) SqCC cases were unclassified. Multiplex stain combinations of Desmoglein + Napsin A, TTF-1 + CK5, and p63 + TRIM29 were comparable in sensitivity and specificity to the

Results cont'd

corresponding single stains (Photo 1-6). With this six antibody panel, grade 1 and 2 LADC and SqCC were classified with 96.4% (135/140) sensitivity, while grade 3 tumors demonstrated 85.7% (60/70) sensitivity (Table 3).

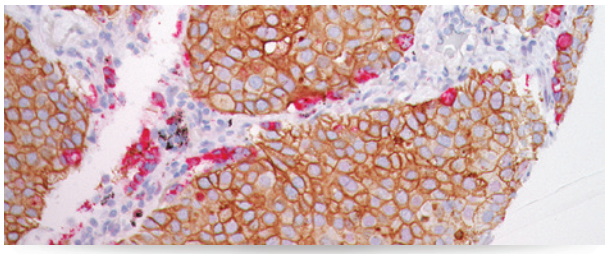
Conclusion

Desmoglein-3, CK5 and Napsin A are highly sensitive and 100% specific. The addition of TTF-1 to Napsin A increases sensitivity for LADC by 6.0% (91.3% overall sensitivity). Co-expression of TTF-1 and Napsin A is considered pulmonary specific, with the exception of some thyroid cancers. TRIM29, a relatively new antibody with limited publications, provided the highest sensitivity (92.6%) in SqCC. The combination of TRIM29 and/or p63 was useful for SqCC classification in several cases. Grade 3 lung cancers appears to be more difficult to classify, and therefore a more extensive panel, such as that described in this study should be considered. The six antibody panel shows great promise in the initial screening of squamous versus adenocarcinoma differentiation. To the best of our knowledge, the six antibody panel provided the highest sensitivity and specificity of any panel previously reported.

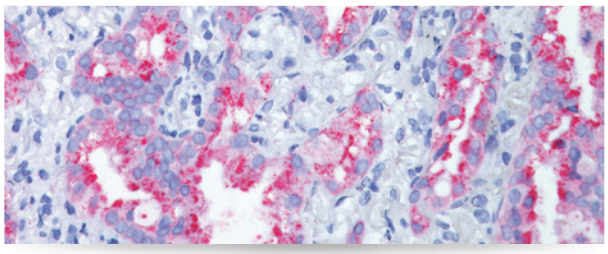
Table 1

Antibodies Tested for Sensitivity and Specificity in this Study	
TTF-1*	Thrombomodulin (CD141)
Napsin A*	34betaE12
Desmoglein 3*	CAM5.2
Cytokeratin 5 (CK5)*	LAT-1 (SLC7A5)
p63*	CEACAM5 [COL-1]
TRIM29*	MUC1 [LL-6]

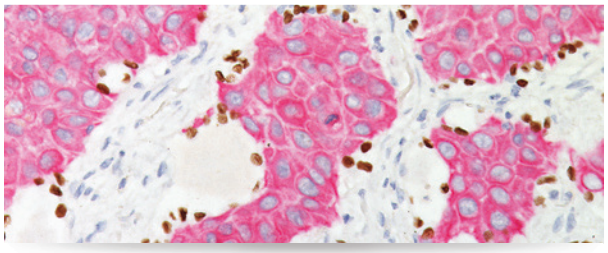
* Antibodies selected based on sensitivity and specificity



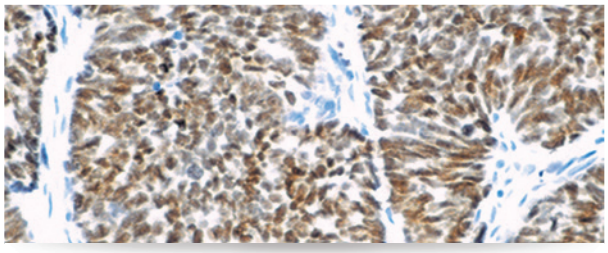
Desmoglein-3 (DAB) + Napsin A (FR) staining lung SqCC



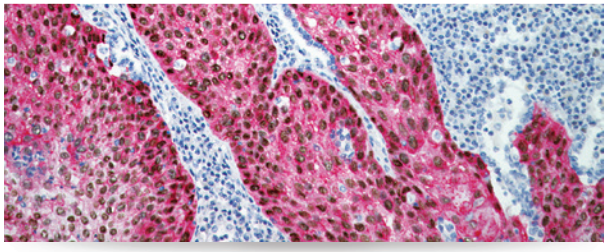
Napsin A staining lung adenocarcinoma



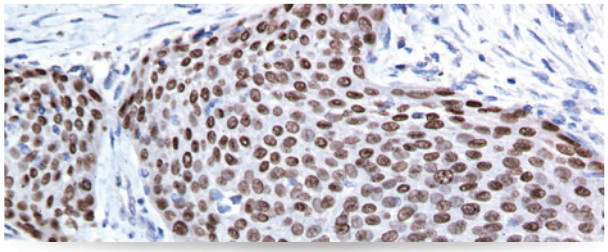
TTF-1 (DAB) + CK5 (FR) staining lung SqCC



TTF-1 staining lung adenocarcinoma



p63 (DAB) + TRIM29 (FR) staining lung SqCC



p63 staining lung SqCC

Table 2: Six Antibody Panel

Antibodies	TTF-1	Napsin A	**Napsin A** and/or **TTF-1**	p63	TRIM29	**p63** and/or **TRIM29**	DSG-3	CK5	**DSG 3** and/or **CK5**
Lung ADC	80/115 70%	99/115 86%	**105/115 91.3%**	13/115 11.3%	8/115 7.0%	**13/115 11.3%**	0/115 0%	0/115 0%	**0/115 0%**
Lung SqCC	5/95 5.3%	0/95 0%	**5/95 5.3%**	84/95 88.4%	88/95 92.6%	**90/95 94.7%**	81/95 85.3%	82/95 86.3%	**88/95 92.6%**

Table 3: Tumor Grade Correlation

Grades	# of cases	% Classified	% Unclassified
1-3	210	195/210 (92.9%)	15/210 (7.1%)
1	29 (14%)	29/29 (100%)	0/29 (0%)
2	111 (53%)	106/111 (95.5%)	5/111 (4.5%)
3	70 (33%)	60/70 (85.7%)	10/70 (14.3%)

References

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