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TTF-1 [SPT24] Staining Specificity in Lung Adenocarcinoma vs. Lung Squamous Cell Carcinoma is Markedly Improved with Titer Optimization and Cut-Off Values

David Tacha, PhD and Ding Zhou, BS; Biocare Medical, Concord, CA

Introduction

Lung cancer is the leading cause of cancer death for both men and women. More people die of lung cancer than of colon, breast and prostate cancers combined.[1] Classification of lung carcinomas into histological types is typically performed by visual examination using Hematoxylin and Eosin (H&E) and immunohistochemistry (IHC). However, accurate classification can be difficult with poorly differentiated or undifferentiated lung carcinoma. Historically, antibodies TTF-1 and p63 have often been used to differentiate primary lung adenocarcinoma (LADC) from squamous cell carcinoma (SqCC) of the lung.[2-4]

Commercially available Thyroid Transcription Factor-1 (TTF-1) monoclonal antibodies 8G7G3/1 and SPT24 have been shown to have different sensitivities in LADC and in lung SqCC.[5-7] A study by Masai *et al.* demonstrated that SPT24 was more sensitive than 8G7G3/1 in LADC (72.4% and 65.4%, respectively).[5] However, the study demonstrated that SPT24 stained a higher percentage of lung SqCC (16.8% vs. 1%). Increased staining of SPT24 in lung SqCC has also been shown to be heavily influenced by different detection systems.[8] These findings with SPT24 led us to investigate antibody dilution factor and staining cut-off values along with additional screening antibodies to provide better specificity. Desmoglein 3 (DSG3) and p40 have recently been shown to be highly specific for lung SqCC while Napsin A has demonstrated absolute specificities in LADC.[4,9,10] Double stain cocktails including DSG3 + p40 as well as TTF-1 + Napsin A have shown high specificity and sensitivity in lung SqCC.[4,10,11] The co-expression of TTF-1 and Napsin A has also been shown to be highly specific for LADC, and its co-expression is regarded as pulmonary specific.[11]

In this study, we will screen cases of lung SqCC and LADC with a double stain cocktail of DSG3/p40 + Napsin A. The SPT24 titer will be optimized for LADC and cut-off values will be examined to improve overall staining specificity without compromising staining sensitivity.

Materials and Methods

Formalin-fixed paraffin-embedded lung SqCC (n=137) and LADC (n=60) tissue microarrays were acquired. DSG3/p40 + Napsin A double stain cocktail (Biocare Medical, Concord, CA), TTF-1 [SPT24] and TTF-1 [8G7G3/1] antibody titers were optimized for IHC. SPT24 was optimally titered at 1:1200. The double stain antibody cocktail was detected by using a polymer double stain detection kit and visualization was achieved with DAB and Fast Red chromogens.

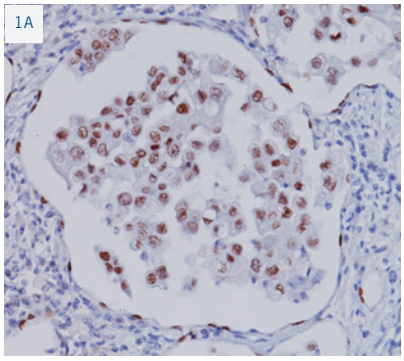
Scoring and Interpretation Method

TTF-1 cases were considered positive if 10% or more of tumor cells were stained with a staining intensity of > 1+. Cases with <10% staining and no focal areas of positive staining were scored as negative.

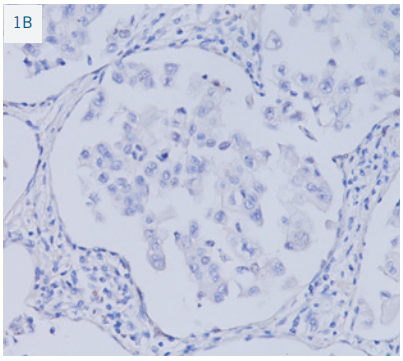
Results

Results are summarized in Tables 1 and 2. TTF-1 [SPT24] and TTF-1 [8G7G3/1] both stained 1.5% (2/137) of lung SqCC (Table 1). Napsin A was negative in all lung SqCC while DSG3/p40 stained 91.2% of lung SqCC (Table 1). In LADC, TTF-1 [SPT24] stained 88.3% of cases compared to Napsin A (73.3%) and TTF-1 [8G7G3/1] (63.3%) (Table 2). Lung SqCC cases that demonstrated expression of SPT24 below the cutoff value (<10%) were positive for DSG3 and/or p40.

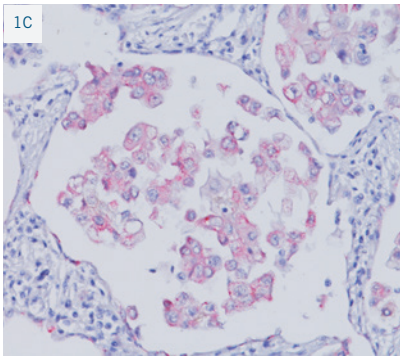
Figure 1



Lung adenocarcinoma (LADC) stained with SPT24

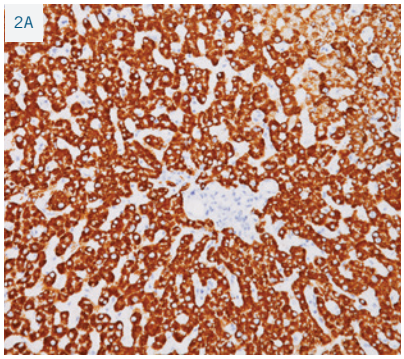


LADC stained with 8G7G3/1

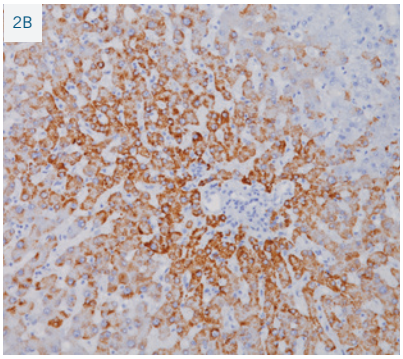


LADC stained with Napsin A

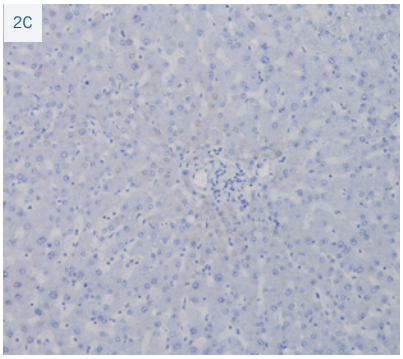
Figure 2



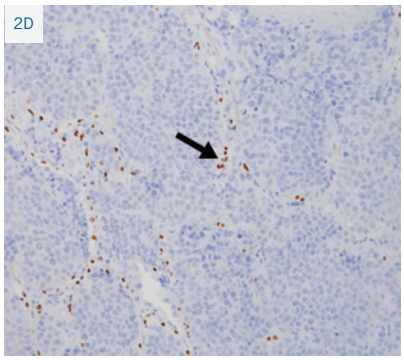
Liver stained with 8G7G3/1



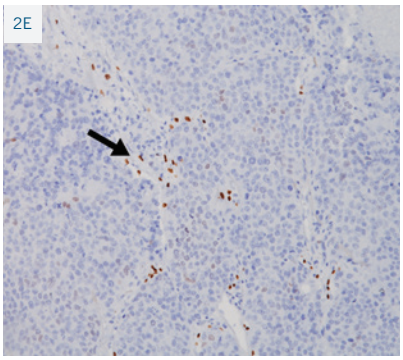
Liver stained with SPT24 1:200



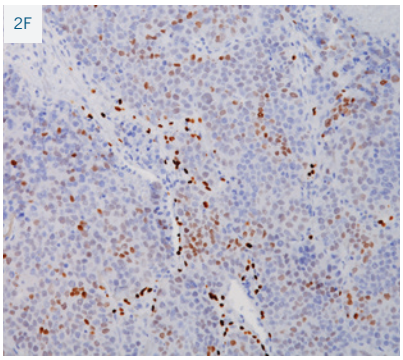
Liver stained with SPT24 1:1200



Lung SqCC stained with 8G7G3/1

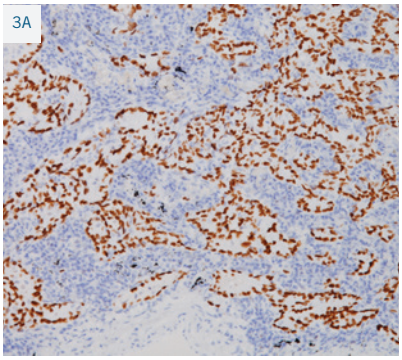


Lung SqCC stained with SPT24 1:1200

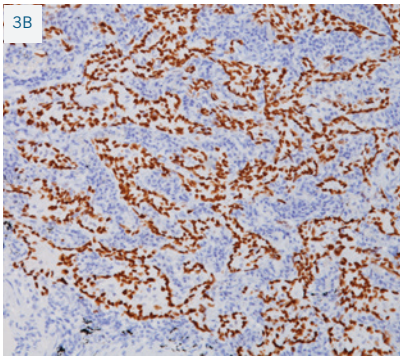


Lung SqCC stained with SPT24 1:200

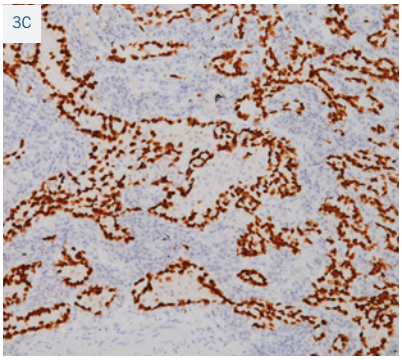
Figure 3



LADC stained with 8G7G3/1



LADC stained with SPT24 1:1200



LADC stained with SPT24 1:200

Table 1

DSG3/p40	Napsin A	TTF-1 [SPT24]	TTF-1 [8G7G3/1]
125/137 (91.2%)	0/137 (0%)	2/137 (1.5%)	2/137 (1.5%)

Table 2

DSG3/p40	Napsin A	TTF-1 [SPT24]	TTF-1 [8G7G3/1]
1/60 (1.7%)*	44/60 (73.3%)	53/60 (88.3%)	38/60 (63.3%)

*One case of lung adenocarcinoma stained with p40 but also stained with Napsin A.

Discussion

The well published use of 8G7G3/1 has been shown to stain lung adenocarcinoma and small cell lung carcinoma but, in most cases, not lung squamous cell carcinoma.[2,3] In this study, SPT24 and Napsin A were shown to be superior at detecting LADC when compared to 8G7G3/1 (Table 2, Figures 1A-C). When applying a wide range of SPT24 antibody titers (1:200-1:1200) on liver and lung cancer, we observed staining in normal liver (cytoplasmic) and in certain cases of lung SqCC when a titer of 1:200 was used. Normal liver and the expression of SPT24 originally observed in lung SqCC were both negative when SPT24 was systematically diluted out to 1:1200 (Figures 2A-F). Only residual normal lung was stained with both TTF-1 antibodies (Arrows, Figure 2D, 2E). Strong and diffuse staining in LADC was achieved with a 1:1200 dilution of SPT24 and staining intensity was comparable to 8G7G3/1 (Figures 3A, 3B). However, at 1:200, SPT24 staining intensity was stronger than the optimized titers of 8G7G3/1 and SPT24 (Figure 3C).

Based on the interpretation method in a study by Mukhopadhyay and Katzenstein, a ≥10% cut-off value for positive staining was used to increase specificity and decrease potential false positives.[12] Correlation of confirmed cases of LADC and lung SqCC was achieved by IHC screening with the double stain cocktail of DSG3/p40 + Napsin A (Tables 1 and 2). Two poorly differentiated cases that had been previously diagnosed as lung SqCC expressed both SPT24 and 8G7G3/1 antibodies but were negative for DSG3, p40 and Napsin A. As a result, we could not confirm the previous diagnosis of lung SqCC by IHC or by morphological examination.

Conclusion

TTF-1 [SPT24] was more sensitive than TTF-1 [8G7G3/1] in LADC. TTF-1 [SPT24] specificity in lung SqCC is significantly improved with titer optimization and cut-off values and has been shown to be equivalent to TTF-1 [8G7G3/1]. When properly titered, TTF-1 [SPT24] does not stain normal liver whereas TTF-1 [8G7G3/1] does stain normal liver. The co-expression of TTF-1 and Napsin A provides further specificity for lung adenocarcinoma or when determining tumors of unknown origin. DSG3 and p40 are highly specific for lung SqCC and therefore, may be used as a pre-screener to rule out LADC. Caution or the use of other confirmatory markers should be considered when poorly differentiated lung cancers are expressing TTF-1 but are negative for DSG3, p40 and Napsin A.

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4040 Pike Lane
Concord CA 94520

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