Human Pathology (2013) **44**, 1479–1486

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Human PATHOLOGY

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Original contribution

p40 (ΔNp63) and keratin 34βE12 provide greater diagnostic accuracy than p63 in the evaluation of small cell lung carcinoma in small biopsy samples☆

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Received 13 December 2012; revised 10 January 2013; accepted 11 January 2013

Keywords:
p40;
ΔNp63;
p63;
Keratin 34βE12;
Small cell lung carcinoma;
Bronchoscopic biopsy;
Immunohistochemistry

Summary The use of p63 has been advocated for separating small cell lung carcinoma from poorly differentiated non–small cell lung carcinoma, in particular, squamous cell lung carcinoma. However, p63 is not entirely specific in this distinction, as several studies have demonstrated p63 immunoreactivity in a proportion of small cell lung carcinomas. p40 (ΔNp63) has recently been purported to be a highly specific marker for squamous cell lung carcinoma, but data regarding p40 (ΔNp63) in small cell lung carcinoma, a key differential diagnostic consideration in biopsy samples of squamous cell lung carcinoma, are lacking. In this study, a total of 34 previously confirmed small cell lung carcinomas (27 bronchoscopic biopsy samples and 7 large specimens) were immunostained for p40 (ΔNp63), p63, and keratin 34βE12. All 34 small cell lung carcinomas were negative for p40 (ΔNp63) and keratin 34βE12. Although none of the large small cell lung carcinoma specimens exhibited p63 immunoreactivity, 12 (44.4%) of 27 biopsy samples of small cell lung carcinoma were positive for p63. The rate of p63 staining in small cell lung carcinoma biopsy samples differed significantly from that of p40 (ΔNp63) and keratin 34βE12 ($P = .005$). Ten cases of squamous cell lung carcinoma were also tested, all of which were positive for all 3 markers. These findings confirm that p63 immunoexpression is not uncommon in biopsy samples of small cell lung carcinoma. Positive p63 staining may be mistakenly interpreted as supportive of squamous differentiation and result in misclassification of small cell lung carcinoma as squamous cell lung carcinoma, a diagnostic error that has important therapeutic and prognostic consequences. To provide greater diagnostic accuracy, p40 (ΔNp63) or keratin 34βE12 should be used instead of p63 in the distinction of small cell lung carcinoma from non–small cell lung carcinoma in biopsy samples.

1. Introduction

With the emergence of targeted chemotherapeutic agents, accurate histologic classification of lung carcinoma is crucial, as decisions regarding testing of tumors for sensitizing molecular alterations are primarily based on histologic subtype. A number of recent studies have

☆ Conflict of interest/financial disclosures: No outside financial support was received for this project. The authors declare that they have no conflicts of interest.

Presented in part at the 102nd annual meeting of the United States and Canadian Academy of Pathology, March 2013, Baltimore, MD, USA.

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0046-8177/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.humpath.2013.01.011

investigated the optimal immunohistochemical (IHC) approach for distinguishing pulmonary adenocarcinoma (ADCA) from squamous cell carcinoma (SQCC) [1–3]. The distinction of small cell lung carcinoma (SCLC) from poorly differentiated non-SCLC (NSCLC) using IHC is a topic that has, by comparison, garnered little recent attention.

SCLC is a diagnosis that, in principle, can be made by light microscopy. With their scant amount of cytoplasm, SCLC tumor cells are prone to crush artifact, which can pose a diagnostic challenge, particularly in bronchoscopic biopsies. Differential diagnostic considerations of SCLC with severe crush artifact include lymphoid proliferations, other tumors within the spectrum of pulmonary neuroendocrine neoplasms, malignant melanoma, primitive neuroectodermal tumor, and some types of metastatic carcinoma as well as various types of NSCLC, most notably poorly differentiated SQCC, the basaloid and small cell variants of SQCC, and basaloid carcinoma.

It was recognized quite some time ago that the wispy perinuclear rim and punctate paranuclear dot-like pattern of immunostaining for cytokeratins Cam 5.2 and AE1/3 exhibited by some cases of SCLC can help eliminate not only lymphoid proliferations and melanoma from diagnostic consideration but also, to some extent, NSCLC [4]. Broad-spectrum cytokeratin staining in NSCLC is typically more intense and has a circumferential cytoplasmic and membranous distribution. However, it should be noted that not all cases exhibit these stereotypic patterns of immunoreactivity [5].

More recently, keratin 34βE12 was shown to have utility in the separation of NSCLC, specifically SQCC and basaloid carcinoma, from SCLC, with the former entities being positive and SCLC being negative for this marker in most cases [6–8]. As additional experience with keratin 34βE12 was gained, it was realized that this marker also stains a substantial proportion (82%) of pulmonary ADCAs [3]. Given that various subtypes of SQCC represent the main NSCLC mimickers of SCLC, some authors have advocated using p63 for this distinction [5].

Although purported to be specific for SQCC, p63 stains a proportion of pulmonary ADCAs (32%), albeit lower than the proportion that stain with keratin 34βE12 [3]. With respect to SCLC, in our own practice, we have encountered a number of cases that stain positively for p63. Although a number of studies have reported p63 staining to be completely absent in SCLC, our observation has been corroborated by several reports in the literature, which describe rates of SCLC p63 immunoreactivity as high as 76.9% (Table) [9–17].

p40 (ΔNp63) has gained recent attention as being a more highly specific marker of squamous differentiation than p63 in the distinction of pulmonary SQCC from ADCA [18–20]. It would be ideal if this marker also aids in the separation of SCLC from NSCLC. However, very limited data have been published on the staining characteristics of p40 (ΔNp63) SCLC [21].

The high sensitivity of p40 (ΔNp63) for SQCC has been established in prior studies [18–20]. In the present study, our primary objective is, therefore, not to reaffirm the sensitivity of p40 (ΔNp63) for SQCC, although the study design includes cases of SQCC to confirm that expected positive staining results are achieved, but rather expand our

Table IHC expression of p63 in SCLC

Study	Specimen types	No. of cases	Antibody clone [a]	Source	Dilution	Antigen retrieval	% p63 positive
Wang et al [9]	Bronchoscopic biopsies and resections	9	4A4	Santa Cruz Biotechnology	–	Citrate	0
Pelosi et al [10]	Resections	10	4A4	Dako	1:500	Citrate	20
Wu et al [11]	Cytology cell blocks and resections	23	4A4	Santa Cruz Biotechnology	1:800	Citrate	0
Au et al [12]	Tissue microarray	13	4A4	Neomarkers	1:1000	Citrate	76.9 30.8 [b]
Zhang et al [13]	Bronchoscopic biopsies and resections	28	7JUL	Novocastra	1:100	EDTA	0
Hiroshima et al [14]	Resections	23	4A4	Dako	1:400	Citrate	4
Kalhor et al [15]	Cytology smears/cell blocks, surgical biopsies, resections	13	7JUL	Novocastra	1:100	EDTA	0
Kargi et al [16]	Bronchoscopic biopsies	28	4A4/Y4A3 cocktail	Neomarkers	1:100	Citrate	0
Maleki [17]	Cytology smears/cell blocks	4	–	–	–	–	75
Present study	Bronchoscopic biopsies Resections/autopsies	27 7	BC4A4	Biocare Medical	1:100	Citrate	44.4 0

Abbreviation: –, indicates information not provided.

[a] All studies used mouse monoclonal (mAb) antibodies.

[b] IHC performed at 2 different institutions.