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Original contribution

Immunohistochemical profiles in primary lung cancers and epithelial pulmonary metastases☆,☆☆

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Summary Correct diagnosis of pulmonary tumors is essential for treatment decision and often relies on immunohistochemical markers. We stained tissue microarrays from resected primary lung cancer (n = 665) and pulmonary metastases (n = 425) for CK7, CK20, CDX2, CK5, p40, p63, TTF-1, napsin A, GATA3, and PAX8 to systematically assess the diagnostic value of these markers. Primary lung adenocarcinomas expressed TTF-1 in 90% and napsin A in 84% of the cases, whereas 10% were positive for p63, 7% for CDX2, 2% for CK20, and 2% for GATA3. Only 68% of the lung adenocarcinomas were positive for CK7, TTF-1, and napsin A and negative for all other markers. Primary lung squamous cell carcinomas expressed CK5, p40, and p63 in 94%-97% of cases, whereas 44% were positive for CK7, 20% for GATA3, 7% for CDX2, and 3% for TTF-1. Rare cases expressed PAX8, CK20, or napsin A. Pulmonary metastases of colorectal cancer were positive for CK20 in 83% and CDX2 in 99% of the cases. Rare cases expressed CK7, p63, or PAX8, whereas 4% expressed TTF-1. Pulmonary metastases of renal cell carcinomas were positive for PAX8 in 74%, napsin A in 7%, and CK7 in 7% of the cases. Pulmonary metastases of breast cancer were positive for GATA3 in 93% and CK7 in 78% of the cases, whereas 15% expressed CK5. Information on expression and patterns of immunohistochemical markers facilitates histopathological diagnostics. Evidently, unusual immune profiles occur and may lead to incorrect diagnosis.

1. Introduction

Determination of histological type and origin of pulmonary tumors is highly important for correct choice of oncological and surgical treatment. Immunohistochemical (IHC) staining aids in histopathological diagnostics of cancer, where IHC

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markers are used alone or, more commonly, in panels to confirm or reject a diagnosis. Because the diagnostics of pulmonary tumors is typically performed on small specimens, IHC staining is often needed.

We have previously investigated 3 different clones of thyroid transcription factor-1 (TTF-1) in primary lung cancers and pulmonary metastases [1], and also napsin A, cytokeratin (CK) 5, p40, and p63 in primary lung cancers [2]. To further assess IHC markers in clinical use systematically, we decided to also investigate CK7, CK20, caudal type homeobox 2 (CDX2), GATA3, and paired box 8 (PAX8).

TTF-1 is highly specific for lung and thyroid adenocarcinomas (ACs) and high-grade neuroendocrine carcinomas [3,4]. Together with TTF-1, napsin A serves as a diagnostic marker to differentiate pulmonary AC from squamous cell carcinoma (SqCC) [5]. Other tumors commonly positive for napsin A include clear cell and papillary renal cell carcinomas (RCCs) [6] and clear cell ovarian and endometrial carcinomas [7,8].

CK5, p40, and p63 are all used as markers of squamous differentiation [9], but CK5 is also expressed in, for example, mesotheliomas, basal-like breast carcinomas, thymomas, and some salivary gland tumors and urothelial carcinomas, the latter 3 typically positive for p40 and p63 as well [10,11].

CK7 and CK20 profile is useful to distinguish, for example, ovarian, pulmonary, and breast carcinomas (CK7+/CK20−) from colon (CK7−/CK20+), urothelial (CK7+/CK20+), and renal and prostatic carcinomas (CK7−/CK20−) [7,8,11-13]. CDX2 is also a useful marker for establishing gastrointestinal origin because most ACs of the colon, small intestines, stomach, and esophagus are CDX2+ [7,8,14,15].

GATA3 is used as a marker for breast and urothelial carcinoma. Additionally, it has been reported to be frequently positive in various epithelial skin tumors, chromophobe RCC, and mesothelioma. Ductal pancreatic and salivary gland ACs as well as some lung cancers may also be positive [16,17]. PAX8 is used as a marker for renal, ovarian, and endometrial cancer [15,18,19] but may also be positive in, for example, thyroid and thymic tumors [18-20].

The aim of the present study was to compare the staining properties of these 10 commonly used IHC markers in primary lung cancers and pulmonary metastases to explore their usefulness in the differential diagnostics of pulmonary tumors.

2. Materials and methods

2.1. Study population

The present study included 665 resected primary lung cancers from 657 individuals (8 cases with 2 synchronous primary lung cancers each) originally included in 3 independent unselective cohorts from the Uppsala Lung Cancer Study, the Southern Swedish Lung Cancer Study, and the Malmö Diet and Cancer Study. All cases were previously updated in accordance with the fourth edition of the WHO classification on lung cancer [2,21], and the results of IHC staining for TTF-1, napsin A, CK5, p40, and p63 have been published elsewhere, as well as some other markers for part of the cases [2,22].

The pulmonary metastases cohort was a retrospective, consecutive study from the Skåne University Hospital in Lund covering the years 2000-2014 [1]. All cases with surgical lung resection of an epithelial malignant tumor consistent with a metastasis were included. The cohort included 440 resected pulmonary metastases from 351 patients. There were 15 metastases with no tumor tissue in the tissue microarray (TMA) slides in the present study; therefore, 425 pulmonary metastases from 342 patients were available for assessment (60 cases each had 2 and 12 cases each had 3 resected metastases to the lungs originating from the same tumor).

As part of the present investigation, we discovered 1 case that was erroneously diagnosed as primary lung SqCC in the clinical setting but proved to be a pulmonary metastasis of the patient's previously known urothelial carcinoma (further IHC markers, such as positive uroplakin II, confirmed the diagnosis). This case has been included as a primary lung SqCC in some of our previous publications [2,22,23], but in the present study, the case was included in the pulmonary metastasis cohort because it fitted the inclusion criteria. In addition, we identified 3 cases erroneously diagnosed as pulmonary metastases of colorectal cancer (CRC) and 1 case as metastasis of endometrial cancer in the clinical setting. Comparisons with the primary tumors and comprehensive IHC panels with distinctly different profiles (including differently expressed CK7, CDX2, CK20, TTF-1, and napsin A, and TTF-1, napsin A, and PAX8, respectively) proved the pulmonary tumors to be separate primary lung cancers, and the cases were consequently excluded from the study and in the numbers above.

2.2. TMA construction

TMAs were constructed with 2 cores, 1 mm in diameter, from each tumor from the Uppsala Lung Cancer Study, the Malmö Diet and Cancer Study, and the pulmonary metastases cohorts, whereas 3 cores were used for each case from the Southern Swedish Lung Cancer Study cohort. For the cohort of pulmonary metastases, cores were taken from each metastasis if several pulmonary metastases had been surgically treated. Also, cores from the primary tumor were included when available (70% of the cases).

2.3. Immunohistochemistry

Four-micrometer–thick tissue sections from the TMAs were automatically pretreated and stained with the IHC markers. Detailed information of the antibodies, pretreatment, and control tissue is found in Table 1. The fraction of positive viable tumor cells was scored using 5 categories: less than 1%,

Table 1 Details for the used immunohistochemical stainings

Antibody	Clone	Vendor	Dilution	Pretreatment	Staining pattern	Positive control	Negative control	Internal positive control [a]
CK7	SP52	Ventana Medical Systems (Tucson, AZ)	RTU	CC1	Cytoplasmic	Liver (bile ducts)	Liver (hepatocytes)	Type II pneumocytes
CK20	SP33	Ventana	RTU	CC1	Cytoplasmic	Appendix	Tonsil, liver	Gastrointestinal ACs
CDX2	EPR2764Y	Ventana	RTU	CC1 + Amp	Nuclear	Pancreas (ducts), small intestine [b]	Tonsil [b]	Gastrointestinal ACs
CK5	XM26	Leica Biosystems (Nussloch, Germany)	1:25	CC1	Cytoplasmic	Tonsil (epithelium)	Liver, appendix	Basal cells
p40	BC28	Histolab/Biocare Medical (Concord, CA)	1:50	CC1	Nuclear	Tonsil (epithelium)	Thyroid, kidney	Basal cells
p63	4A4	Ventana	RTU	CC1 + Amp.	Nuclear	Tonsil (epithelium)	Thyroid, kidney	Basal cells
TTF-1	SPT24	Leica	1:50	CC1 + Amp.	Nuclear	Thyroid	Tonsil, kidney	Type II pneumocytes, terminal bronchioles
Napsin A	IP64	Leica	1:20	CC2	Cytoplasmic	Kidney (proximal tubules)	Tonsil, thyroid	Type II pneumocytes, alveolar macrophages
GATA3	L50-823	Cell Marque (Rocklin, CA)	1:50	CC1 + Amp	Nuclear	Kidney (collecting ducts), tonsil (T lymphocytes)	Tonsil (B lymphocytes), thyroid	T lymphocytes
PAX8	MRQ-50	Cell Marque	RTU	CC1 + Amp	Nuclear	Kidney, thyroid	Muscle including blood vessels	Lymphocytes (scattered)

Abbreviations: Amp, amplification; CC1, Ventana Cell Conditioning 1 (EDTA, pH 8); CC2, Ventana Cell Conditioning 1 (citrate, pH 6); RTU, ready to use.

[a] Internal control here denotes cell types that were present on all slides but not for all individual cases.

[b] For about half of the slides, either tonsil was missing as negative control or appendix was used as positive control.

1%-9%, 10%-24%, 25%-49%, and 50% or more. Special care was taken not to interpret, for example, trapped alveolar or bronchiolar epithelium or macrophages as tumor cells.

For adenosquamous carcinoma and mixed large cell neuroendocrine carcinoma (LCNEC), the 2 cell populations were evaluated separately. The AC component of these cases was immunohistochemically similar to "pure" pulmonary AC and therefore grouped together with the AC cases. The same was valid for the SqCC component of the adenosquamous carcinomas and "pure" pulmonary SqCC. For the adenosquamous cancers from cervix, only the AC component was present in the pulmonary metastases. For tumors with intermingled cell populations such as adenoid cystic cancer and malignant myoepithelioma, all tumor cells were evaluated as a single component. For thymomas, only the epithelial cells were evaluated.

Cytoplasmic staining for napsin A, CK5, CK7, and CK20 and nuclear staining for TTF-1, CDX2, p40, p63, GATA3, and PAX8 were considered positive. Results for TTF-1 were taken from our previous investigation [1]. For the pulmonary metastasis cohort, the remaining markers were evaluated independently by a PhD student (H. V.) and a pathologist (H. B.), with cases scored differently discussed for consensus. For the primary lung cancer cohorts, the markers were evaluated by a pathologist (H. B.).

2.4. Ethics

The analyses of the cohorts were conducted in adherence to the Declaration of Helsinki and were approved by the regional ethical review boards in Uppsala (Dnr 2012/532) and Lund (Dnr 2004/762 and 2008/702 and Dnr 2007/445, 2008/35, and 2014/748, respectively).

3. Results

Histological types—and subtypes of potential relevance—for the included 665 primary lung cancers and 425 pulmonary

Table 2 Frequency (percent) of positive primary lung cancers for different immunohistochemical stains presented as 1% or more/10% or more positive tumor cells

Tumor type	No. of tumors	CK7	CK20	CDX2	CK5	p40	p63	TTF-1	Napsin A	GATA3	PAX8
AC [a]	431	99/99	4/2	11/7	0.5/0.5	2/0.2	26/10	93/90	88/84	4/2	0.5/0
Solid predominant	81	94/94	5/4	6/4	2/2	5/1	23/10	86/86	69/63	6/2	1/0
Mucinous	21	100/100	29/10	43/29	0/0	0/0	5/0	57/38	62/33	10/0	0/0
SqCC [b]	202	46/44	2/1	13/7	97/96	97/94	98/97	6/3	2/0.5	30/20	2/2
Large cell carcinoma	9	67/67	0/0	56/56	11/0	0/0	22/0	0/0	0/0	22/0	25/13
Sarcomatoid carcinoma [c]	6	83/83	0/0	0/0	0/0	17/0	17/17	50/50	33/33	17/17	0/0
Small cell carcinoma	3	33/33	0/0	0/0	0/0	0/0	0/0	33/33	0/0	0/0	67/33
LCNEC	21	62/57	0/0	38/24	5/0	0/0	5/0	86/81	0/0	0/0	5/0
Carcinoid tumor	7	86/57	0/0	0/0	0/0	0/0	0/0	86/71	0/0	0/0	0/0

[a] Including the AC component of 14 cases with mixed histology (8 adenosquamous carcinomas and 6 combined LCNECs).
[b] Including the SqCC component of 8 cases with mixed histology (adenosquamous carcinomas).
[c] Five pleomorphic carcinomas with an AC component and 1 giant cell carcinoma.

metastases are found in Tables 2 and 3, respectively. The tables summarize the results of IHC expression for CK7, CK20, CDX2, CK5, p40, p63, TTF-1, napsin A, GATA 3, and PAX8 in the primary lung cancers and pulmonary metastases, respectively. In both tables, results are shown for ≥1% and ≥10% positive tumor cells as cutoff for a positive staining. More detailed information is found in Supplementary Tables 1 and 2, respectively. For facilitated comparisons and presentation, a positive staining hereafter refers to ≥10% positive tumor cells unless otherwise specified (including the text below and all figures).

Co-occurrence of selected IHC markers in primary lung AC and SqCC and in pulmonary metastases of CRC is presented in Fig. 1. The typical pattern for lung AC was CK7+/TTF−1+/napsin A+. However, only 68% of the cases expressed all these 3 and no other of the evaluated markers. Correspondingly, 64% of lung SqCC cases were CK5+/p40+/p63+ and CK7+/− and negative for all other markers.

Table 3 Frequency (percent) of positive epithelial pulmonary metastases for different immunohistochemical stains presented as 1% or more/10% or more positive tumor cells

Tumor origin or type	No. of tumors	CK7	CK20	CDX2	CK5	p40	p63	TTF-1	Napsin A	GATA3	PAX8
Colorectal carcinoma [a]	277	3/2	91/83	99/99	1/0	0/0	0.7/0.4	7/4	0/0	0/0	0.7/0.7
RCC [b]	42	10/7	0/0	2/2	0/0	0/0	0/0	0/0	10/7	2/2	86/74
Breast carcinoma [c]	27	78/78	0/0	0/0	19/15	7/4	19/7	0/0	0/0	93/93	4/0
Gynecological carcinomas [d]	17	71/71	6/6	53/41	6/6	0/0	6/6	12/0	0/0	29/24	71/71
Prostatic carcinoma	11	0/0	9/0	55/36	0/0	0/0	0/0	0/0	0/0	0/0	9/0
SqCC [e]	11	18/18	0/0	60/50	100/100	100/100	100/100	0/0	0/0	36/36	18/0
Urothelial carcinoma	8	100/100	50/50	25/13	38/0	100/100	100/100	13/13	0/0	100/100	0/0
Adenoid cystic carcinoma [f]	6	100/100	0/0	0/0	100/100	100/100	100/100	0/0	0/0	33/17	0/0
Thymoma [g]	5	40/40	0/0	0/0	100/100	80/80	80/80	0/0	0/0	0/0	80/60
Pancreatic carcinoma	5	100/100	60/40	80/40	0/0	0/0	0/0	20/0	0/0	0/0	20/20
Appendix carcinoma	4	0/0	100/100	100/100	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Hepatocellular carcinoma	4	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Thyroid carcinoma [h]	3	67/67	0/0	0/0	0/0	0/0	33/0	67/67	0/0	33/0	67/67
Small bowel carcinoma	2	0/0	100/100	100/100	0/0	0/0	0/0	50/0	0/0	50/0	0/0
Cholangiocarcinoma	1	100/100	0/0	100/100	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Esophageal AC	1	100/100	100/0	100/100	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Basal cell carcinoma	1	0/0	0/0	0/0	100/100	100/100	100/100	0/0	0/0	100/100	0/0

NOTE. When not specified, cases were ACs with morphological features typical of site of origin (eg, prostatic and gastrointestinal carcinomas).
[a] All ACs (whereof 18 mucinous) with gland formations and/or cribriform pattern with intestinal features.
[b] Thirty-three clear cell, 4 papillary, and 5 other/indeterminate ACs.
[c] Twenty-six ACs of ductal/no special type (whereof 1 mixed mucinous) and 1 malignant adenomyoepithelioma.
[d] Six from uterus (5 endometroid ACs, 1 carcinosarcoma), 8 from cervix (6 ACs and 2 AC component of adenosquamous carcinomas), 2 from ovarium (1 clear cell and 1 mucinous AC), and 1 AC from vulva.
[e] Four from tonsil, 3 from anus, 2 from esophagus, 1 from uterine cervix, and 1 from oral cavity.
[f] Four from salivary glands and 2 from vulva.
[g] One type B1 and 4 type B3.
[h] One each of papillary, follicular, and anaplastic AC.

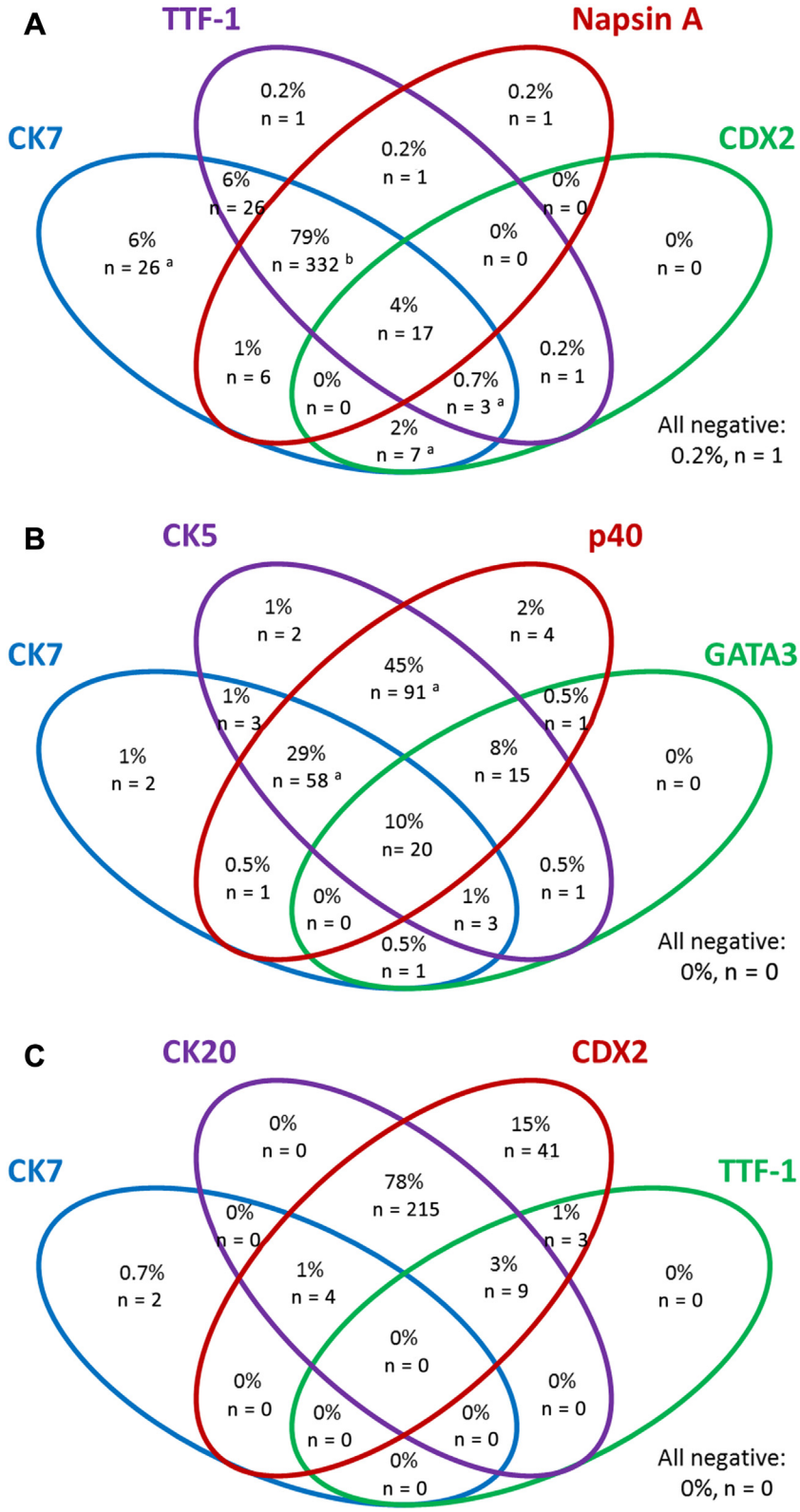

Fig. 1 Immunohistochemical profiles with ≥10% positive tumor cells defining a positive staining. A, Primary lung ACs, 422 cases including AC component of 14 mixed tumors (9 cases with missing data for CDX2 are omitted in the figure). Notes *a* and *b* indicate that 1 and 6 cases, respectively, in this group were positive for CK20. B, Primary lung SqCCs, 201 cases including squamous component of 7 adenosquamous carcinomas (1 case with missing data for GATA3 is omitted). Note *a* indicates that 1 case in this group was positive for CK20. C, Pulmonary metastases of colorectal ACs, 274 cases (3 cases with missing data for CK7 are omitted). All cases were negative for napsin A. The 2 metastases positive only for CK7 were from the same patient.

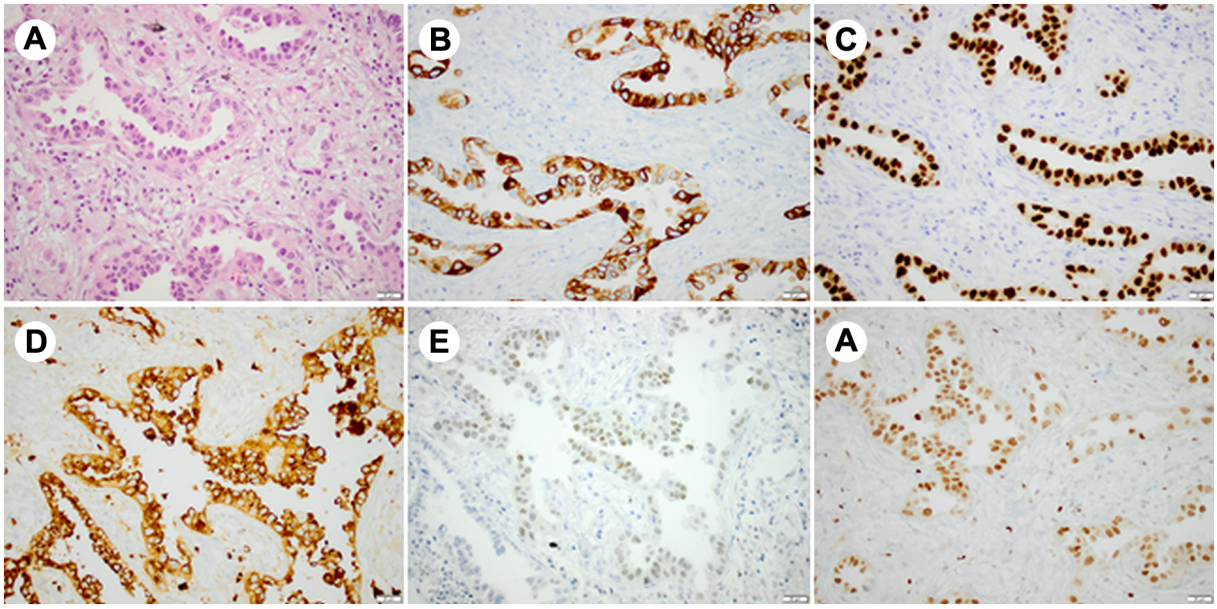

Fig. 2 A case of lung AC positive for both GATA3 and CDX2 (weakly). A, Hematoxylin-eosin, (B) CK7, (C) TTF-1, (D) napsin A, (E) CDX2, and (F) GATA3. Scale bar is 20 μm.

Pulmonary metastases of CRC were CK20+/CDX2+ in 78%, whereas those of RCC were PAX8+ and napsin A+/− in 71% and those of breast cancer were CK7+/GATA3+ in 59% of the cases while being negative for unmentioned markers.

3.1. CK7, CK20, and CDX2

Co-occurrence of CK7, CK20, and CDX2 in lung AC is presented in Fig. 1A, and co-occurrence of CK7 and CK20 in lung SqCC is in Fig. 1B. Example of a CDX2+ lung AC and a CK20+ lung SqCC is seen in Figs. 2 and 3, respectively, whereas representative CDX2 staining is also seen in Fig. 4. One lung AC was positive for both CK20 and CDX2 (and also CK7) and at the same time negative for TTF-1 and napsin A (Fig. 1A). This case was an invasive mucinous AC. All of the 13 CDX2+ lung SqCC cases were CK20−, whereas 5 were CK7+ (all 13 were CK5+/p40+). For both pulmonary large cell carcinoma and LCNEC cases, 3 of 5 CDX2+ cases were CK7+, respectively.

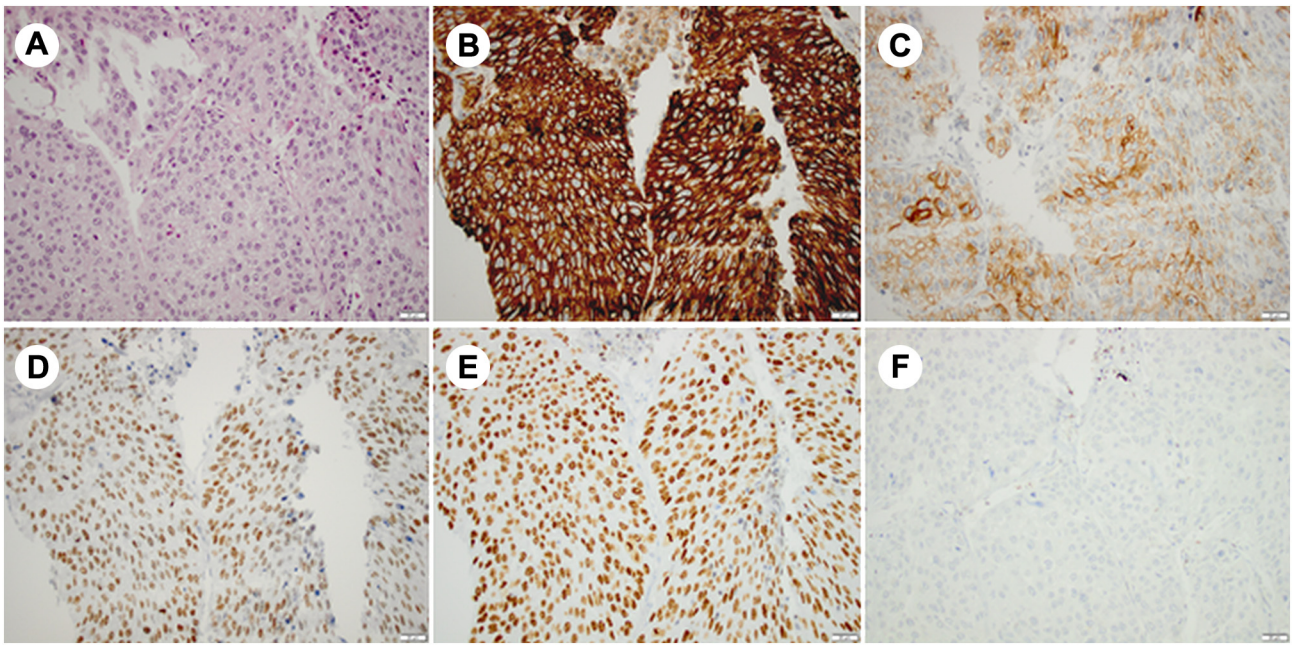

Fig. 3 A case of lung SqCC positive for CK20. A, Hematoxylin-eosin, (B) CK5, (C) CK20, (D) p40, (E) p63, and (F) GATA3. Scale bar is 20 μm.

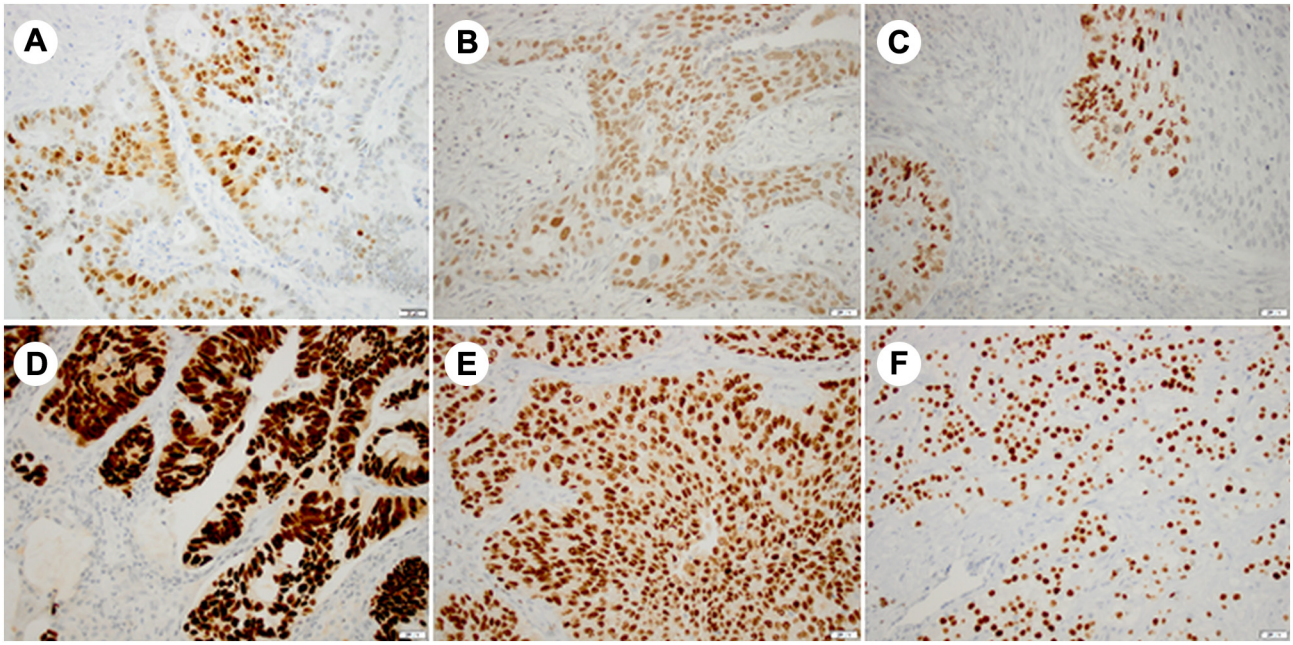

Fig. 4 Comparison of typical staining intensities for (A and D) CDX2, (B and E) GATA3, and (C and F) PAX8 in positive (A) lung AC, (B and C) lung SqCC and pulmonary metastases of (D) CRC, (E) urothelial carcinoma, and (F) RCC, respectively. Scale bar is 20 μm.

For most types of pulmonary metastases, the number of cases with co-occurrence of any of CK7, CK20, and CDX2 may be concluded from Table 3 and, for CRC, Fig. 1C. Five of the 6 CK7+ and 30 of the 46 CK20− metastases of CRC originated from the rectum. The 2 CK7+/CK20−/CDX2− metastases of rectal cancer from the same patient have been previously described (this case was PAX8+ and on a whole tumor slide also focally TTF-1+) [24].

The CDX2+ metastasis of RCC was also CK7+ (papillary RCC; all other markers were negative). One metastasis of mucinous AC from vulva was CK7−/CK20+/CDX2+, and 1 endometrial AC of endometroid type was CK7−/CK20−/CDX2+. Five other gynecological AC of various origin were CK7+/CK20−/CDX2+ and 7 (including the AC component of 2 adenosquamous cancers) were CK7+ only, whereas 2 ACs and the carcinosarcoma metastasis were negative for all 3 markers. The 5 CDX2+ pulmonary SqCC metastases, including 3 metastases of tonsillar cancer from the same patient and 2 esophageal cancers, were all CK7−, whereas the 2 CK7+ cases (both CDX2−) originated from the anus. The CDX2+ metastasis of urothelial carcinoma was also CK20+. Among the metastases of pancreatic cancer, 1 each was CK20+/CDX2−, CK20−/CDX2+, and CK20+/CDX2+, whereas 2 cases were negative for both markers.

3.2. CK5, p40, and p63

All p40+ cases were also p63+. Co-occurrence of CK5 and p40 in lung SqCC is presented in Fig. 1B. One of the 3 CK5−/p40− SqCCs (all 3 were CK7+) was p63+ (and GATA3+), whereas another case expressed p63 only in <10% of the cells. There were 2 CK5+ lung ACs, both solid predominant, whereof 1 was p40+/p63+ and the other p40−/p63+ (both were positive for CK7 and TTF-1 but only 1 for napsin A).

The single p40+ breast cancer metastasis was a malignant epithelial-myoepithelial carcinoma, which also exhibited CK5 positivity but only in <10% of the cells (CK7 was negative and GATA3 positive). This case has been described previously [24]. There were 4 CK5+ metastases of breast cancer (all ductal/no special type), whereof 1 was also p63+. One metastasis of endometrial AC (endometroid type) was CK5+, whereas p40 and p63 were negative. One other gynecological cancer, a cervical adenosquamous carcinoma (the pulmonary metastasis exhibiting only glandular differentiation), was positive for p63 only.

3.3. TTF-1 and napsin A

Co-occurrence of TTF-1 and napsin A in primary lung AC is found in Fig. 1A. As evident, 83% of lung AC expressed both and 92% at least 1 of the 2 markers. If using ≥1% positive tumor cells as cutoff for a positive staining instead, 372 of 431 lung ACs (86%) expressed both and 406 of 431 (94%) either of TTF-1 and napsin A. The corresponding numbers for mucinous lung AC was 11 (52%) and 14 (67%) of 21 cases and for solid predominant lung AC 55 (68%) and 71 (88%) of 81 cases. The 2 napsin A+ sarcomatoid carcinomas were both TTF-1+ (both were pleomorphic carcinomas with an AC component). There was no other case with co-occurrence of TTF-1 and napsin A (also true if ≥1% positive tumor cells were used as cutoff for a positive staining). The TTF-1+ metastasis of urothelial carcinoma was positive for CK7, CK20, p40, p63, and GATA3.

3.4. GATA3

Most pulmonary metastases of breast cancer and all metastases of urothelial carcinoma were GATA3+ (Table 3). The 2 GATA3− breast cancer metastases were both CK7+, and 1 of them was also CK5+ (the primary tumor had the same profile), whereas another CK7+/CK5+ breast cancer metastasis was positive for GATA3 in only 10%-24% of the cells (all these 3 cases were p40−/p63−).

Other GATA3+ pulmonary metastases included 4 cervix cancers (3 ACs from the same patient and the AC component of 1 adenosquamous carcinoma), 3 SqCCs from the tonsil (all from the same patient), and 1 each of adenoid cystic cancer from the vulva, SqCC from the anus, RCC (partial GATA3+, CK7+, PAX8−; uncertain if oncocytic papillary or chromophobe type, no surgery of primary tumor for comparison), and basal cell carcinoma.

Co-occurrence of GATA3 with CK7, CK5, and/or p40 in lung SqCC is found in Fig. 1B. Of the 9 GATA3+ lung AC cases, 3 were positive for both TTF-1 and napsin A, 2 were negative for both markers, and 3 were positive for TTF-1 only and 1 for napsin A only (all 9 cases were CK7+). Example of a GATA3+ lung AC is seen in Fig. 2, whereas representative GATA3 staining is also shown in Fig. 4.

Using ≥50% positive tumor cells as cutoff for a positive GATA3 staining, 85% (23 of 27) of pulmonary breast cancer metastases and 100% of urothelial cancer metastases were positive, whereas only 6 other pulmonary metastases and 26 primary lung cancers were positive (Supplementary Tables 1 and 2).

3.5. PAX8

As evident from Table 3, pulmonary metastases of RCC, gynecological cancer, thymoma, and thyroid cancer were commonly PAX8+. Of the 11 PAX8− RCC metastases (whereof 8 were clear cell RCC), all were napsin A− and 2 were CK7+ (whereof 1 was CDX2+ and papillary, and 1 was GATA3+ and papillary or chromophobe). Only 1 of the 33 PAX8+ RCCs was CK7+ (morphology suggesting clear cell carcinoma), whereas 3 other cases were napsin A+. The 5 PAX8- gynecological cancer metastases were AC (2 cases) and adenosquamous cancer from cervix, AC from vulva, and endometrial carcinosarcoma. Two of the 12 PAX8+ gynecological cancers were CK7− (both endometrial AC of endometroid type). One of the 2 PAX8− thymoma metastases (type B3) was CK5+/p40−/p63− and also CK7− (all other thymomas were CK5+/p40+/p63+). Two thyroid cancer metastases were positive for PAX8, CK7, and TTF-1, whereas 1 case of anaplastic thyroid cancer was negative for all evaluated IHC stains.

Other tumors were rarely positive for PAX8. The 2 metastases of PAX8+ rectal cancer (both from the same patient) have been described above and previously [24]. The PAX8+ metastasis of pancreatic cancer was positive for CK7, CK20, and CDX2. All 3 PAX8+ primary lung SqCCs were CK5+/p40+ (2 were also TTF-1+, whereof 1 was CK7+). The PAX8+ small cell carcinoma was negative for all other markers, whereas the PAX8+ large cell carcinoma was positive for CK7 only. Representative PAX8 staining is seen in Fig. 4.

4. Discussion

We here present staining patterns of clinically useful diagnostic IHC markers in primary lung cancers and pulmonary metastases. The focus on co-occurrence of markers more resembles the clinical situation where IHC panels are often used. Expression of individual markers not typical for a specific histopathological diagnosis was uncommon, but altogether an IHC profile that was deviant from the typical one occurred quite frequently. Some deviant patterns may not cause much problem in the clinical setting, such as positive CDX2 in a TTF-1+/napsin A+ lung AC or in a CK5+/p40+ lung SqCC. However, for example, CK20+ lung SqCC, CK7− lung AC (especially if also negative TTF-1), and CK7+ CRC are potentially much more problematic. Lung SqCC may have the same IHC profile as urothelial carcinoma and basal-like breast cancer, and on small specimens, these diagnoses may also be difficult to separate based on morphology.

Our results show that CDX2 is more sensitive but less specific than CK20 for gastrointestinal origin. This is in line with previous studies on primary and metastatic CRC and other primary gastrointestinal cancers, although all primary lung ACs have been negative for CDX2 in some studies [25-27]. GPA33 is a new antibody (not evaluated in the present study) that has been suggested to be as sensitive as but more specific than CDX2 for CRC [27].

In our study, GATA3 was infrequently positive in several tumor types other than breast and urothelial cancer. This is in line with the study by Miettinen and coworkers [16], whereas GATA3 was almost perfectly specific in the study by Liu and coworkers [17]. Most notably, a rather high proportion of lung SqCC was GATA3+ in our study, higher than reported by Miettinen and coworkers, although the latter reported more positive lung AC cases than we did [16]. The cases of our study have been thoroughly reviewed regarding morphology and stained with multiple markers for correct histological diagnosis, which is why we believe that it is correct that GATA3 is more often positive in lung SqCC than AC.

PAX8 was positive in slightly fewer cases of RCC metastases in our material than generally reported in the literature, but many previous studies have evaluated primary RCC and used a polyclonal antibody that may be more sensitive and less specific [15,19,20,28]. Also, fewer RCCs were napsin A+ in our study (7%) than in, for example, the study by Xu and coworkers [6], where 39% of primary and 34% of metastatic clear cell RCCs were napsin A+. However, other studies have reported series of napsin A− pulmonary metastases of RCC and loss of expression in metastases compared to primary

tumors, which may be an explanation [29,30]. Our finding is strengthened by the expression of napsin A in primary lung AC in our material, which was in line with other studies [5].

The main strengths of the present study are the large number of cases included, that we used pulmonary metastases instead of nonpulmonary primary tumors, and that we have analyzed combinations of multiple markers to evaluate IHC profiles. The main limitations are the use of TMA instead of whole sections (although more resembling biopsies) and the low number of some types of primary lung cancers and most types of pulmonary metastases (the cohorts representing surgically treated cases).

In conclusion, the results of our study provide knowledge that aid in diagnostics of a pulmonary tumor. Nontypical IHC expression is quite common, and unusual immune profiles occur and may lead to an incorrect diagnosis. For example, SqCC may have the same IHC profile as basal-like breast or urothelial carcinoma. Also, lung ACs that are TTF-1−/napsin A− have the same immune profile as several other tumor forms and in exceptional cases the same as CRC.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.humpath.2018.10.009.

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Author contributions

H. V. coevaluated part of the stainings, analyzed the results, and wrote the manuscript. L. T. performed the stainings. B. N. constructed the TMAs. K. J., M. P., P. J., J. S. M. M., J. B., P. M., and H. B. participated in cohort creation. H. B. designed the study, reevaluated the cases (some together with P. M.), evaluated the stainings, analyzed the results, and assisted in manuscript writing. All authors reviewed the manuscript for its scientific content.

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