Value of p63 and Cytokeratin 5/6 as Immunohistochemical Markers for the Differential Diagnosis of Poorly Differentiated and Undifferentiated Carcinomas

Olaf Kaufmann, MD, Ellen Fietze, MD, Jörg Mengs, and Manfred Dietel, MD

Key Words: p63; Cytokeratin 5/6; Poorly differentiated carcinomas; Immunohistochemistry

Abstract

To facilitate the differential diagnosis of poorly differentiated metastatic carcinomas of unknown primary site, we evaluated p63 and cytokeratin (CK) 5/6 as immunohistochemical markers for squamous cell carcinomas. The study cases were as follows: squamous cell carcinoma of the lungs, head/neck, esophagus, cervix uteri, or anal canal, 73; non–squamous cell carcinomas of various primary sites, 141; and urothelial carcinoma, 20. We also tested 14 malignant mesotheliomas.

Immunoreactivity for p63 was as follows: squamous cell carcinomas, 59 (81%); urothelial carcinoma, 14 (70%), most often with diffuse staining patterns; non–squamous cell carcinomas, 20 (14.2%), resulting in a specificity of 0.86 of p63 for squamous cell carcinomas. Coexpression of p63 and CK5/6 had a sensitivity of 0.77 and a specificity of 0.96 for squamous cell carcinomas. Increasing the minimal criterion of positive immunostaining for both markers to more than 50% of immunoreactive tumor cells resulted in a specificity of 0.99, although the sensitivity diminished to 0.66. All malignant mesotheliomas were negative for p63.

Our data suggest that positive immunostaining for both p63 and CK5/6 in poorly differentiated metastatic carcinomas is highly predictive of a primary tumor of squamous epithelial origin.

Metastatic carcinomas of unknown primary site represent about 2% to 5% of all newly diagnosed carcinomas.[1,2] Light microscopic examination reveals that about 30% of carcinomas of unknown primary site are poorly differentiated or undifferentiated carcinomas.[1] Within this heterogeneous tumor group, only extragonadal germ cell tumors and neuroendocrine carcinomas are treated by chemotherapy, irrespective of their primary site.[3] In contrast, knowing the primary site of somatic nonneuroendocrine carcinomas of unknown primary site would be of clinical importance, as patients then could be treated according to protocols that are specific for advanced stages of the respective carcinoma types. Furthermore, lacking knowledge of the primary tumor site poses an additional psychological burden[4,5] on patients and their families.

The immunohistochemical identification of primary carcinoma sites usually is based on the detection of more or less organ-specific terminal differentiation products or transcription factors. Most of these markers are commercially available and substantially facilitate the identification of primary sites of metastatic adenocarcinomas (for a recent review see Hammar[4]), but often have a low sensitivity in poorly differentiated carcinomas. Therefore, instead of using organ-specific markers, in poorly differentiated carcinomas it might be more rewarding to use markers that are associated with minimal "histogenetic" differentiation. For instance, the expression of markers specifically associated with squamous differentiation limits the possible primary site of a carcinoma for practical purposes to only a few locations (head/neck, lungs, esophagus, cervix uteri).

Since the mid-1980s,[6,7] commercially available monoclonal antibodies recognizing basal cell–type high-molecular-weight cytokeratins (CKs) 5 and 14 according to the catalog

Table 1
Immunoreactivity for p63

Tumor	No. of Cases	Percentage of Positive Cells				Total No. (%) of Positive Cases
		0	1-10	11-50	>50	
Poorly differentiated squamous cell carcinoma						
Head/neck	32	6	2	2	22	24 (75)
Esophagus	8	0	0	0	8	8 (100)
Cervix uteri	15	2	0	1	12	13 (87)
Anal canal	3	1	0	0	2	2 (67)
Lungs	15	3	0	1	11	12 (80)
Total	73	12	2	4	55	59 (81)
Non–squamous cell carcinoma						
Colon	13	12	0	1	0	1 (8)
Pancreas	7	4	1	1	1	2 (29)
Biliary tract	5	4	0	1	0	1 (20)
Renal cell	13	12	0	1	0	1 (8)
Stomach	13	10	1	2	0	2 (15)
Breast	28	18	7	2	1	3 (11)
Ovaries	15	10	3	1	1	2 (13)
Prostate	8	7	1	0	0	0 (0)
Hepatocellular	9	8	0	1	0	1 (11)
Embryonal	9	9	0	0	0	0 (0)
Lungs						
Adenocarcinoma	12	5	2	3	2	5 (42)
Small cell	9	7	0	1	1	2 (22)
Total	141	106	15	14	6	20 (14.2)
Urothelial carcinoma	20	3	3	2	12	14 (70)
Mesothelioma	14	12	1	0	0	0 (0)

by Moll et al[8] were established as the most sensitive, although not entirely specific, paraffin-reactive markers associated with a squamous differentiation in carcinomas. The recently cloned transcription factor p63 is another promising marker to indicate a minimal squamous differentiation in a poorly differentiated carcinoma. The *p63* gene is located at chromosome 3q27-29 and belongs to the *p53* gene family.[9] The *p63* gene encodes multiple isotypes with divergent abilities to transactivate *p53* reporter genes and induce apoptosis. Importantly, the predominant p63 isotypes in most epithelial tissues lack the N-terminal transactivating domain and act in a dominant-negative manner regarding the transactivating effects of p53 and other p63 isotypes.[9] In normal tissues, p63 was reported to be immunohistochemically detectable in basal cells of all squamous epithelia (including epidermis and hair follicles), in basal cells of urothelium, and in basal cells of prostate epithelium.[9,10] Furthermore, the truncated isotype p63 is detectable in most squamous cell carcinomas (including undifferentiated nasopharyngeal carcinomas) of various primary sites.[10-13] The p63 overexpression in these tumors is apparently due to an amplification of the *p63* gene.[12,13]

In the present study, we evaluated the potential significance of p63 as an immunohistochemical marker for poorly differentiated metastatic squamous cell carcinomas. We used the commercially available paraffin-reactive monoclonal antibody 4A4 against p63 and compared the results with immunostaining for CK5/6 using monoclonal antibody D5/16B4.

Materials and Methods

Selection of Cases

The surgical pathology files of the Charité University Hospital, Berlin, Germany, were surveyed to retrieve representative paraffin blocks of a total of 248 metastases, the primary tumors of which had been classified as poorly differentiated (grade 3) or undifferentiated (grade 4) carcinomas **Table 1** and that practically could result in a finding of carcinoma of unknown primary site. The selected grade 3 carcinomas included only cases showing minimal differentiation features that were most prevalent in the respective tumor site, eg, poorly differentiated carcinomas of the cervix comprised only squamous cell carcinomas, but not adenocarcinomas. Very small specimens and needle biopsy specimens were not included.

Since all squamous cell carcinomas were of the nonkeratinizing type, their morphologic classification, by definition, depended on the presence of intercellular bridges. Unfortunately, the presence or absence of intercellular bridges is difficult to verify in poorly differentiated carcinomas, especially in metastatic infiltrates, without knowledge of the primary tumor site. Therefore, grade 3 squamous cell carcinomas and grade 4 carcinomas of the same primary sites were grouped together and were classified as poorly differentiated squamous cell carcinomas for practical purposes. Tumors that

Table 2
Immunoreactivity for Cytokeratin 5/6

Tumor	No. of Cases	Percentage of Positive Cells				Total No. (%) of Positive Cases
		0	1-10	11-50	>50	
Poorly differentiated squamous cell carcinoma						
Head/neck	32	6	1	3	22	25 (78)
Esophagus	8	0	0	2	6	8 (100)
Cervix uteri	15	1	1	3	10	13 (87)
Anal canal	3	0	1	0	2	2 (67)
Lungs	15	1	1	2	11	13 (93)
Total	73	8	4	10	51	61 (84)
Non–squamous cell carcinoma						
Colon	13	12	0	1	0	1 (8)
Pancreas	7	5	1	1	0	1 (14)
Biliary tract	5	3	1	1	0	1 (20)
Renal cell	13	12	1	0	0	0 (0)
Stomach	13	11	1	1	0	1 (8)
Breast	28	6	5	10	7	17 (61)
Ovaries	15	7	3	4	1	5 (33)
Prostate	8	8	0	0	0	0 (0)
Hepatocellular	9	9	0	0	0	0 (0)
Embryonal	9	9	0	0	0	0 (0)
Lungs						
Adenocarcinoma	12	8	0	4	0	4 (33)
Small cell	9	9	0	0	0	0 (0)
Total	141	99	12	22	8	30 (21.3)
Urothelial carcinoma	20	7	6	5	2	7 (35)
Mesothelioma (10 epithelioid, 4 biphasic)*	14	0	0	2	12	14 (100)

* In the biphasic mesotheliomas, only the immunoreactivity of epithelioid tumor areas was evaluated.

were coded as grade 3 adenocarcinomas and grade 4 carcinomas of the same primary site also were grouped together. Large cell undifferentiated carcinomas of the lung were excluded as, in contrast with undifferentiated carcinomas of other primary sites, this tumor group is very heterogeneous and encompasses carcinomas that show glandular, squamous, or neuroendocrine features by electron microscopy.[14,15] Five of the selected 9 pulmonary small cell carcinomas had been classified as intermediate-type small cell carcinoma according to the "old" World Health Organization histologic typing of lung tumors[16] and showed no immunoreactivity for synaptophysin and chromogranin A. All other tumors diagnosed as undifferentiated carcinomas were immunostained for synaptophysin and chromogranin A to exclude neuroendocrine differentiation. Furthermore, we included 14 malignant mesotheliomas (13 primary tumors, 1 metastasis) of the pleura (10 cases), the pericardium (1 case), and the peritoneum (3 cases). The mesotheliomas were studied to test whether expression of p63 can be used to discriminate metastatic squamous cell carcinomas and mesotheliomas.

Immunohistochemical Analysis

Deparaffinized 3 to 5 µm sections were rehydrated, and heat-induced epitope retrieval was done by a pressure cooker method[17] in a 10-mmol/L concentration of sodium citrate buffer (Sigma Chemie, Deisenhofen, Germany), pH 6.0. Endogenous avidin-binding activity was blocked as described by Miller and Kubier.[18]

Sections were incubated with the monoclonal antibodies 4A4 against p63 (dilution 1:500; Santa Cruz Biotechnology, Santa Cruz, CA) and D5/16B4 against CK5/6 (dilution 1:200, Zymed Laboratories, San Francisco, CA) for 60 minutes at room temperature. The bound primary antibody was then detected by a secondary biotinylated antibody and a streptavidin-peroxidase conjugate according to the instructions of the manufacturer (Super Sensitive System, BioGenex, San Ramon, CA). Nova Red (Vector, Burlingame, CA) was used as substrate.

Immunostaining results were evaluated semiquantitatively according to the percentage of positive tumor cells, ie, 1% to 10%, 11% to 50%, and more than 50%. Furthermore, we scored whether immunoreactive tumor cells were diffusely or heterogeneously distributed. To exclude equivocal reactions, at least moderate staining intensity in more than 10% of the tumor cells was registered as a diagnostically relevant positive reaction. Only cytoplasmic staining signals were scored as positive reactions for anti-CK5/6. For anti-p63, only a nuclear staining signal was considered a positive reaction.

To calculate the specificity and sensitivity of immunostaining results for squamous cell carcinomas, all tumors were grouped together as specified in Table 1, **Table 2**, and **Table 3** and designated as follows: (1) all squamous cell carcinomas

Table 3
Coexpression of p63 and Cytokeratin 5/6

Tumor	No. of Cases	Coexpression of Markers*	
		>10% of Cells	>50% of Cells
Poorly differentiated squamous cell carcinoma			
Head/neck	32	23 (72)	19 (59)
Esophagus	8	8 (100)	6 (75)
Cervix uteri	15	12 (80)	10 (67)
Anal canal	3	2 (67)	2 (67)
Lungs	15	11 (73)	11 (73)
Total	73	56 (77)	48 (66)
Non–squamous cell carcinoma			
Colon	13	0 (0)	0 (0)
Pancreas	7	0 (0)	0 (0)
Biliary tract	5	0 (0)	0 (0)
Renal cell	13	0 (0)	0 (0)
Stomach	13	0 (0)	0 (0)
Breast	28	2 (7)	0 (0)
Ovaries	15	1 (7)	1 (7)
Prostate	8	0 (0)	0 (0)
Hepatocellular	9	0 (0)	0 (0)
Embryonal	9	0 (0)	0 (0)
Lungs			
Adenocarcinoma	12	2 (17)	0 (0)
Small cell	9	0 (0)	0 (0)
Total	141	5 (3.5)	1 (0.7)
Urothelial carcinoma	20	6 (30)	1 (5)
Mesothelioma	14	0 (0)	0 (0)

* Data are given as number (percentage).

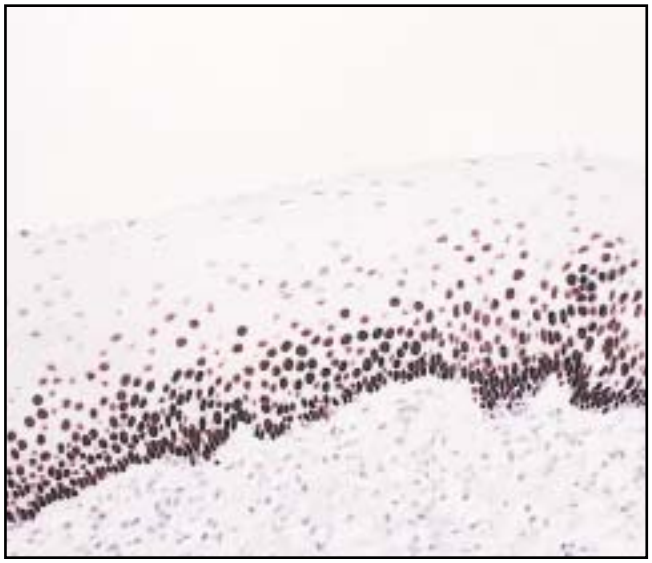

Image 1 Diffuse nuclear immunoreactivity for p63 in basal and intermediate cells of ectocervical squamous epithelium (original magnification ×187).

(including undifferentiated carcinomas of the same primary sites), (2) non–squamous cell carcinomas, (3) urothelial carcinomas, and (4) mesotheliomas. Specificity and sensitivity for squamous cell carcinomas vs non–squamous cell carcinomas (excluding urothelial carcinomas) were calculated as follows:

Specificity = True-Negative Results/True-Negative Results + False-Positive Results

Sensitivity = True-Positive Results/True-Positive Results + False-Negative Results

The designations *true* and *false* are based on the study hypothesis that p63 and CK5/6 are expressed in all squamous cell carcinomas but not in non–squamous cell carcinomas.

Results

Immunoreactivity for p63 in Normal Tissues

In normal tissues, p63 could be detected in basal and intermediate urothelial and squamous cells **Image 1**. Furthermore, basal cells of pseudostratified columnar epithelia (prostate, bronchial epithelium), reserve cells of simple columnar epithelia (focal in endocervical and pancreatic ductal epithelium), germinative cells of sebaceous gland, and all myoepithelial cells (breast, bronchial and oropharyngeal/nasopharyngeal mucous glands, cutaneous eccrine and apocrine glands, major salivary glands) showed strong immunoreactivity for p63. Other epithelial cells and neuroendocrine cells were negative for p63. Ovarian oocytes, but not testicular germ cells, strongly expressed p63. In nonepithelial tissues, p63 was detectable in some lymphoid cells in lymph nodes and extranodal infiltrates. Furthermore,

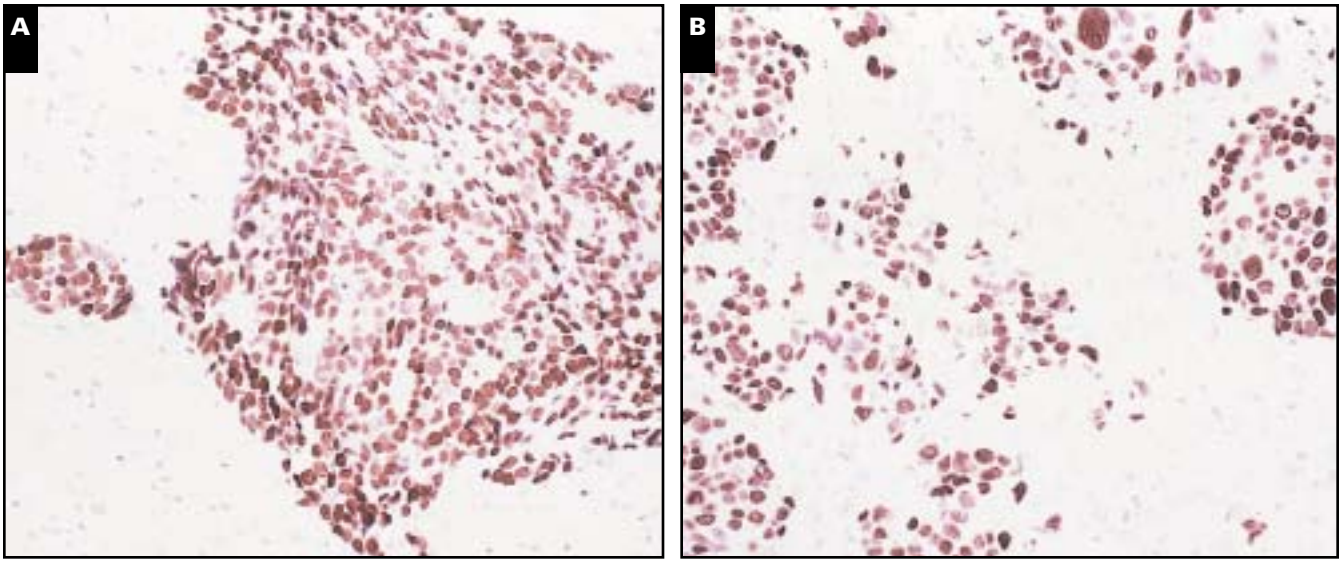

Image 2 Metastatic poorly differentiated squamous cell carcinomas of the larynx (**A**) and the lung (**B**) with diffuse immunoreactivity for p63 (original magnification ×187).

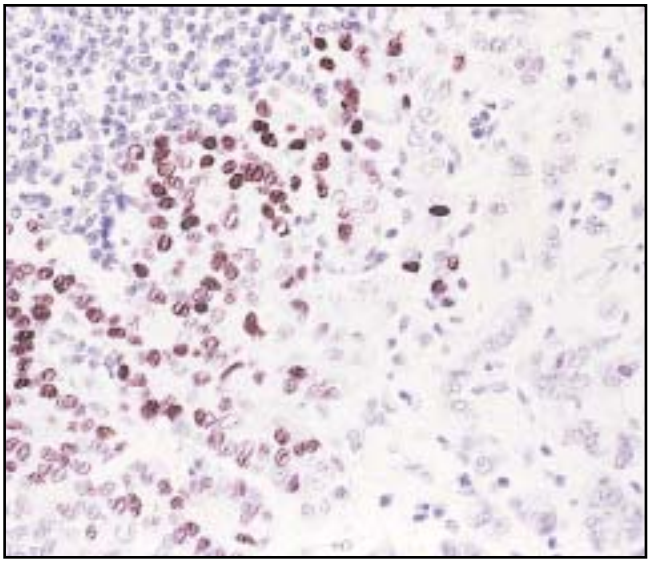

Image 3 Metastatic gastric carcinoma showing a heterogeneously distributed immunostaining for p63 (original magnification ×300).

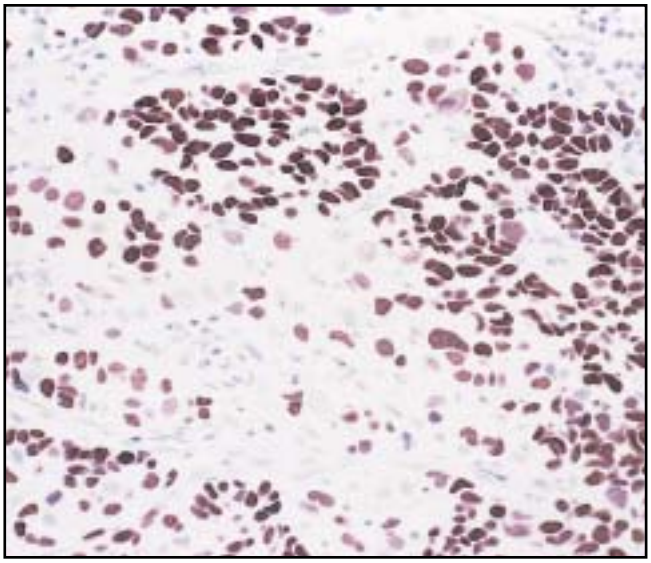

Image 4 Diffuse immunoreactivity for p63 in a metastatic urothelial carcinoma (original magnification ×247).

some reactive pleural and peritoneal mesothelial cells were weakly positive for p63. In mesenchymal tissues, only skeletal muscle fibers showed a consistent strong, although predominantly cytoplasmic, p63 immunoreactivity.

Immunoreactivity for p63 in Tumors

Results of immunostaining for p63 are listed in Table 1. Most squamous cell carcinomas showed diffuse nuclear immunoreactivity for p63 **Image 2**, resulting in a sensitivity of 0.81. Only 20 (14%) of all non–squamous cell carcinomas were positive for p63 in more than 10% of tumor cells, most of them (14/20) with 11% to 50% immunoreactive tumor cells, often with heterogeneous staining patterns **Image 3**. The resulting specificity of p63 for squamous cell carcinomas vs non–squamous cell carcinomas was 0.86. The only carcinoma type showing a majority of p63-positive cases (14/20 [70%]) was urothelial carcinoma **Image 4**. Malignant mesotheliomas were consistently negative for p63.

Immunoreactivity for CK5/6 in Tumors

The results of CK5/6 immunostaining are specified in Table 2. The sensitivity of CK5/6 immunostaining for squamous cell

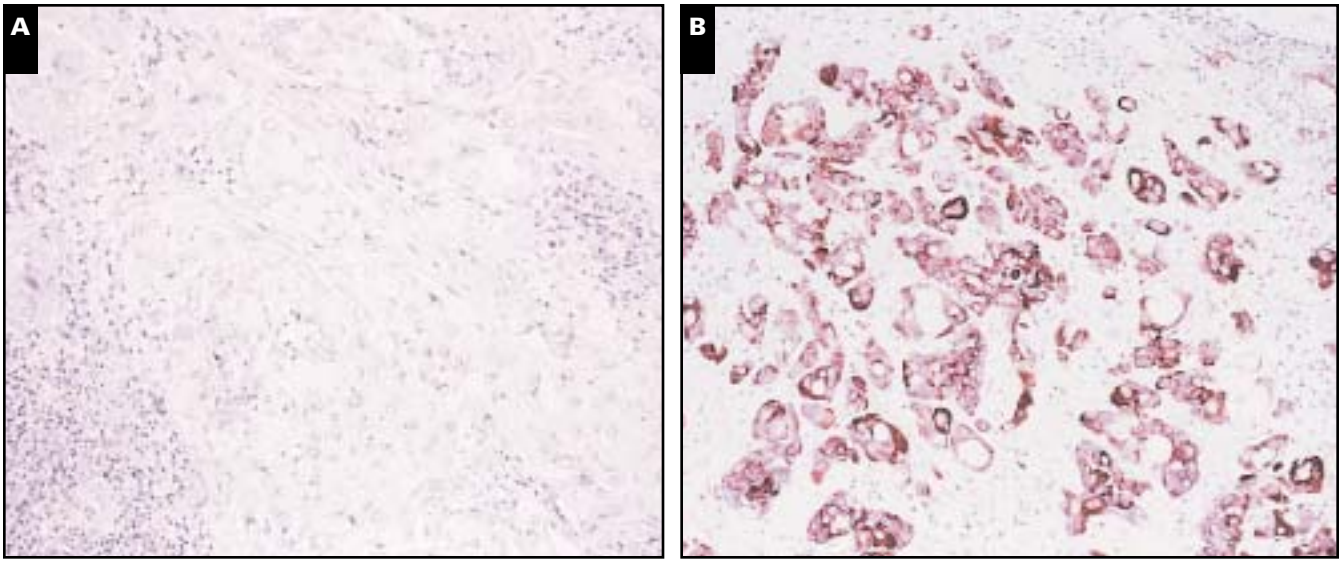

Image 5 Lymph node metastasis of a carcinoma of the breast without immunostaining for p63 (**A**), but showing a diffuse immunoreactivity for cytokeratin 5/6 (**B**) (original magnification ×124).

carcinomas was 0.84. CK5/6 showed positive immunoreactivity in 30 (21.3%) of all non–squamous cell carcinomas, resulting in a specificity for squamous cell carcinomas of 0.79. The most striking difference between p63 and CK5/6 immunostaining results could be seen in carcinomas of the breast, which were positive for CK5/6 in 17 (61%) of 28 cases compared with 3 (11%) of 28 cases positive for p63 **Image 5**. Similar to p63, most (22/30) of the CK5/6-positive non–squamous cell carcinomas showed immunoreactivity in fewer than 50% of tumor cells. Furthermore, 7 (35%) of 20 urothelial carcinomas and all malignant mesotheliomas were positive for CK5/6.

Coexpression of p63 and CK5/6 in Tumors

Most squamous cell carcinomas showed positive immunoreactivity for both p63 and CK5/6 (Table 3). Only 8 cases were positive for one marker but not for the other (5 cases CK5/6-positive/p63-negative; 3 cases CK5/6-negative/p63-positive). The coexpression of both markers in more than 10% of tumor cells had a specificity of 0.96 and a sensitivity of 0.77. The coexpression of both markers in more than 50% of tumor cells reached a specificity of 0.99 for squamous cell carcinomas but was associated with a decline in the sensitivity to 0.66.

Discussion

We tested whether the immunohistochemical detection of p63 alone and in combination with CK5/6 could be used to identify poorly differentiated and undifferentiated metastatic carcinomas of primary sites that are typical for squamous cell carcinomas and to distinguish them from poorly differentiated and undifferentiated carcinomas of other primary sites. In agreement with other authors,[9-11] we detected p63 in basal and intermediate cells of all squamous and urothelial epithelia. Furthermore, myoepithelial cells and basal cells of pseudostratified epithelia were always strongly positive for p63. The expression of p63 in myoepithelial and basal cells is of certain diagnostic interest. Together with other myoepithelial and basal cell markers (especially high-molecular-weight CK and smooth muscle myosin), p63 could be used to verify or to exclude invasion in carcinomas of the breast or the prostate.[19]

In the studied carcinomas, we found p63 in 59 (81%) of all 73 squamous cell carcinomas, usually with diffuse, strong immunoreactivity in the majority of tumor cells. In contrast, only 20 (14.2%) of all non–squamous cell carcinomas (except urothelial carcinomas) were p63-positive, most often in the minority of tumor cells. The detection of p63 in squamous cell carcinomas apparently does not depend on tumor grade, as Crook et al[11] described strong immunoreactivity for p63 even in 25 of 25 undifferentiated nasopharyngeal carcinomas.

Results of immunostaining for CK5/6 in squamous cell and non–squamous cell carcinomas were similar to p63 results. Anti-CK5/6 was only slightly more sensitive (0.84 vs 0.81) and slightly less specific (0.79 vs 0.86) than anti-p63 for squamous cell carcinomas. Nevertheless, an important difference between p63 and CK5/6 immunostaining could be seen in carcinomas of the breast, which were positive for CK5/6 in 61% of the cases (17/28), whereas p63 was detectable in only 11% of the cases (3/28) in more than 10%

of tumor cells. The unusually high rate of CK5/6-positive breast carcinomas in our material is apparently due to the fact that poorly differentiated and undifferentiated carcinomas of the breast significantly more often express basal cell–type high-molecular-weight CK.[20,21]

The specificity of p63 and CK5/6 immunostaining for squamous cell carcinomas could be substantially increased to 0.96, if positive immunoreactivity for both p63 and CK5/6 was used as the minimum criterion to diagnose squamous cell carcinoma. The sensitivity of a coexpression diminished only slightly, to 0.77, compared with p63 (sensitivity, 0.81) and CK5/6 (sensitivity, 0.86) alone. The specificity of a coexpression of both markers in more than 50% of tumor cells reached 0.99, with only 1 undifferentiated ovarian carcinoma as a false-positive case. Unfortunately, the sensitivity then dropped perceptibly to 0.66.

Nevertheless, by using one or both markers with different criteria for positive immunostaining, it is possible to create a kind of continuum for specificity and sensitivity that can be adapted to the clinical situation, as the predictive value of an organ-specific or differentiation-specific marker depends on the a priori or pretest probability of the carcinoma type that is predicted by the marker. Thus, a high predictive value for a squamous cell carcinoma can be achieved with anti-CK5/6 alone (less specific, more sensitive) if a tumor site that usually gives rise to a squamous cell carcinoma has a high pretest probability. In contrast, without any clinical suspicions regarding the primary tumor site, it could be necessary to use a highly specific (alas, less sensitive) marker combination (p63 and CK5/6 with more than 50% of immunoreactive tumor cells as the minimum criterion for positivity) to achieve a high predictive value for a squamous cell carcinoma. If carcinoma of the breast is a relevant differential diagnosis, p63 should also be preferred to CK5/6. Besides squamous cell carcinomas, urothelial carcinomas were the only other type of carcinomas with a p63 positivity in the majority of cases. As a substantial minority of urothelial carcinomas also are positive for CK5/6[22] (35% of cases [7/20] in the present study), other markers than p63 and CK5/6 are necessary for the immunohistochemical discrimination of urothelial and squamous cell carcinomas. Uroplakin III (Progen, Heidelberg, Germany) and/or CK20 are especially useful for this differential diagnostic problem, as they are detectable in most urothelial carcinomas, but almost never in squamous cell carcinomas of different primary sites.[23-27] Although more sensitive, CK7 is a less specific marker for urothelial carcinomas than are uroplakin III and CK20, as squamous cell carcinomas of various primary sites have been reported to be positive.[25,28,29] Chu et al[25] found CK7 positivity in 13 (87%) of 15 squamous cell carcinomas of the cervix uteri; they also found CK7 positivity in 8 (27%) of 30 squamous cell carcinomas of the head and neck region and in 3 (21%) of 14 squamous cell carcinomas of the esophagus. Lyda and Weiss[28] found CK7 positivity in 12 (32%) of 37 squamous cell carcinomas. Wieneke et al[29] found CK7 positivity in 10 (71%) of 14 basaloid squamous cell carcinomas of the sinonasal tract.

The non–squamous cell carcinomas that we studied comprise tumors that most often give rise to metastases of unknown primary site. Nevertheless, it should be kept in mind that we did not study rarer carcinomas that also express p63, eg, carcinomas with a myoepithelial differentiation or sebaceous carcinomas (unpublished data). Therefore, if these tumor types enter the differential diagnosis on clinical and/or morphologic grounds, p63 cannot be used as a marker for a squamous differentiation.

In our material, p63 was not detectable in 14 tested mesotheliomas. Therefore, it can be used to distinguish pleural or peritoneal metastases of nonkeratinizing squamous cell carcinomas from primary solid malignant mesotheliomas. This is of potential diagnostic interest as the mesothelial markers thrombomodulin and CK5/6 that are recommended for the discrimination of mesotheliomas and adenocarcinomas usually also are positive in squamous cell carcinomas.[22,30,31]

Our data suggest that anti-p63 and anti-CK5/6 should be used together to identify about 70% to 80% of all poorly differentiated squamous cell carcinomas and to discriminate them from other poorly differentiated and undifferentiated carcinomas with a specificity of more than 0.95. Therefore, anti-p63 and anti-CK5/6 might be useful components of antibody panels for the immunohistochemical analysis of poorly differentiated metastatic carcinomas of unknown primary site.

From the Institute of Pathology, Charité University Hospital, Berlin, Germany.

Address reprint requests to Dr Fietze: Institute of Pathology, Charité University Hospital, Schumannstr. 20/21, 10117 Berlin, Germany.

References

1. Hainsworth JD, Greco FA. Treatment of patients with cancer of an unknown primary site. *N Engl J Med*. 1993;329:257-263.
2. Muir C. Cancer of unknown primary site. *Cancer*. 1995;75:353-356.
3. Greco FA, Hainsworth JD. Cancer of unknown primary site. In: DeVita VT, Hellman S, Rosenberg SA, eds. *Cancer: Principles and Practice of Oncology*. Philadelphia, PA: Lippincott-Raven; 1997:2423-2443.
4. Hammar SP. Metastatic adenocarcinoma of unknown primary origin. *Hum Pathol*. 1998;29:1393-1402.
5. Hegewisch-Becker S, Hossfeld DK. Metastasen bei unbekanntem Primärtumor. *Onkologe*. 1997;3:338-341.

6. Moll R. Cytokeratins as markers of differentiation in the diagnosis of epithelial tumors. *Subcell Biochem*. 1998;31:205-262.
7. Gown AM, Vogel AM. Monoclonal antibodies to human intermediate filament proteins, III: analysis of tumors. *Am J Clin Pathol*. 1985;84:413-424.
8. Moll R, Franke WW, Schiller DL, et al. The catalog of human cytokeratins: patterns of expression in normal epithelia, tumors and cultured cells. *Cell*. 1982;31:11-24.
9. Yang A, Kaghad M, Wang Y, et al. p63, a p53 homolog at 3q27-29, encodes multiple products with transactivating, death-inducing, and dominant-negative activities. *Mol Cell*. 1998;2:305-316.
10. Parsa R, Yang A, McKeon F, et al. Association of p63 with proliferative potential in normal and neoplastic human keratinocytes. *J Invest Dermatol*. 1999;113:1099-1105.
11. Crook T, Nicholls JM, Brooks L, et al. High level expression of deltaN-p63: a mechanism for the inactivation of p53 in undifferentiated nasopharyngeal carcinoma (NPC)? *Oncogene*. 2000;19:3439-3444.
12. Hibi K, Trink B, Patturajan M, et al. AIS is an oncogene amplified in squamous cell carcinoma. *Proc Natl Acad Sci U S A*. 2000;97:5462-5467.
13. Yamaguchi K, Wu L, Caballero OL, et al. Frequent gain of the p40/p51/p63 gene locus in primary head and neck squamous cell carcinoma. *Int J Cancer*. 2000;86:684-689.
14. Delmonte VC, Alberti O, Saldiva PH. Large cell carcinoma of the lung: ultrastructural and immunohistochemical features. *Chest*. 1986;90:524-527.
15. Leong AS. The relevance of ultrastructural examination in the classification of primary lung tumours. *Pathology*. 1982;14:37-46.
16. The World Health Organization Histological Typing of Lung Tumours, Second Edition. *Am J Clin Pathol*. 1982;77:123-136.
17. Norton AJ, Jordan S, Yeomans P. Brief, high-temperature heat denaturation (pressure cooking): a simple and effective method of antigen retrieval for routinely processed tissues. *J Pathol*. 1994;173:371-379.
18. Miller RT, Kubier P. Blocking of endogenous avidin-binding activity in immunohistochemistry: the use of egg whites. *Appl Immunohistochem*. 1997;5:63-66.
19. Signoretti S, Waltregny D, Dilks J, et al. p63 is a prostate basal cell marker and is required for prostate development. *Am J Pathol*. 2000;157:1769-1775.
20. Malzahn K, Mitze M, Thoenes M, et al. Biological and prognostic significance of stratified epithelial cytokeratins in infiltrating ductal breast carcinomas. *Virchows Arch*. 1998;433:119-129.
21. Tot T. The cytokeratin profile of medullary carcinoma of the breast. *Histopathology*. 2000;37:175-181.
22. Ordonez NG. Value of cytokeratin 5/6 immunostaining in distinguishing epithelial mesothelioma of the pleura from lung adenocarcinoma. *Am J Surg Pathol*. 1998;22:1215-1221.
23. Kaufmann O, Volmerig J, Dietel M. Uroplakin III is a highly specific and moderately sensitive immunohistochemical marker for primary and metastatic urothelial carcinomas. *Am J Clin Pathol*. 2000;113:683-687.
24. Moll R, Wu XR, Lin JH, et al. Uroplakins, specific membrane proteins of urothelial umbrella cells, as histological markers of metastatic transitional cell carcinomas. *Am J Pathol*. 1995;147:1383-1397.
25. Chu P, Wu E, Weiss LM. Cytokeratin 7 and cytokeratin 20 expression in epithelial neoplasms: a survey of 435 cases. *Mod Pathol*. 2000;13:962-972.
26. Miettinen M. Keratin 20: immunohistochemical marker for gastrointestinal, urothelial, and Merkel cell carcinomas. *Mod Pathol*. 1995;8:384-388.
27. Wang NP, Zee S, Zarbo RJ, et al. Coordinate expression of cytokeratins 7 and 20 defines unique subsets of carcinomas. *Appl Immunohistochem*. 1995;3:99-106.
28. Lyda MH, Weiss LM. Immunoreactivity for epithelial and neuroendocrine antibodies are useful in the differential diagnosis of lung carcinomas. *Hum Pathol*. 2000;31:980-987.
29. Wieneke JA, Thompson LD, Wenig BM. Basaloid squamous cell carcinoma of the sinonasal tract. *Cancer*. 1999;85:841-854.
30. Ordonez NG. Thrombomodulin expression in transitional cell carcinoma. *Am J Clin Pathol*. 1998;110:385-390.
31. Ordonez NG. Value of thrombomodulin immunostaining in the diagnosis of mesothelioma. *Histopathology*. 1997;31:25-30.

HOLOGIC®

First and Only FDA Cleared
Digital Cytology System

Genius™ Cervical AI

Genius™ Review Station

Genius™ Digital Imager

Empower Your Genius With Ours

Make a Greater Impact on Cervical Cancer
with the Advanced Technology of the
Genius™ Digital Diagnostics System

Click or Scan
to discover more

genius™
DIGITAL DIAGNOSTICS