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Review

Cytokeratins 20 and 7 as biomarkers: usefulness in discriminating primary from metastatic adenocarcinoma

T. Tot*

Department of Pathology and Clinical Cytology, Central Hospital, Falun, S-791 82 Falun, Sweden

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Abstract

Metastatic adenocarcinoma from an unknown primary site is a common clinical problem. The use of cytokeratins 20 (CK20) and 7 (CK7) was proposed to identify the primary sites in this situation. In this review, the results of 29 studies were summarised and the difficulties of data comparison described. Most tumours retained the CK20 phenotype during metastasis, but lung, non-mucinous ovarian, and gastric adenocarcinomas showed statistically significant differences in CK20 expression in the reported primary and metastatic cases. Ductal breast carcinomas, lung and non-mucinous ovarian adenocarcinomas showed significant differences in CK7 expression when primary and metastatic tumours were compared. CK20 positivity alone indicates metastatic spread of adenocarcinoma in several organs. CK7 negativity is consistent with metastases of adenocarcinomas in the lungs, ovaries, liver or serous membranes. CK20/7 phenotyping of adenocarcinomas is a useful diagnostic tool if based on algorithmic and probabilistic approaches and a detailed database.

Keywords: Metastatic adenocarcinomas; Cytokeratins; Cytokeratin 20; Cytokeratin 7

1. Introduction

Metastatic adenocarcinoma from an unknown primary site is a common clinical problem that leads to extensive and costly clinical and radiological examinations, sometimes with disappointing results [1]. Cytokeratin phenotyping, especially CK20 and CK7, has been proposed to assist and direct the clinical and radiological efforts [2–4]. Cytokeratin phenotyping also may be beneficial when examining patients with more than one known primary tumours. A proper diagnosis of the primary site is important not only for therapeutic decision-making, but also for correct epidemiological registration, which, in turn, influences our knowledge about the natural history and prognosis of particular tumour types. In this era of unacceptably low autopsy rates and fragmentation of pathology into molecular, surgical and anatomical disciplines, the routine use of biomarkers may be useful for correctly determining the primary tumour site.

In this review, I summarised the results of 29 studies [2–30] containing more than 3500 reported cases of adenocarcinomas stained with CK20 and CK7. After pointing out the difficulties in comparing the results, I focused on the possibility of discriminating primary from metastatic adenocarcinomas in the organs, which are the usual sites of metastases.

2. The problems

Identifying relevant publications for a review article is a problematic issue without widely accepted strategies and methodology [31]. The studies included in the present review were partly identified by a Medline search (keyword CK20, keyword CK7, limited to the period 1995–1999) and completed by the basic publications before 1995 cited in most of the selected papers. Studies published in the English language and studies with a representative number of cases were preferred. Individual case reports were excluded. The results of four of my own studies were additionally included. The difficulties in applying the proposed general criteria for identifying relevant publications, e.g. patient selection, technical and statistical differences are described below.

The studies analysed in this review are heterogeneous in many aspects. The influence of technical factors

* Tel.: +46-23-492-696; fax: +46-23-492-389.
E-mail address: tibor.tot@ltdalarna.se (T. Tot).

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(fixation time, type of pretreatment and type of primary antibody) is well known and discussed elsewhere [32]. It is worth mentioning that several studies used an automated system (Ventana, Tucson, AZ, USA) that makes these studies more comparable.

The interpretation of positivity is the next crucial problem. Ten of the analysed studies [4,5,11,14,15,18,22–24,29] considered cases positive if any staining was detectable in the tumour cells. Four studies [2,13,16,27] had a 5% threshold, another six [4,6,8,9,17,20] a 10% threshold, and one study a 50% threshold [27]. One study [7] used a more complicated grading system. In another study, the staining intensity was the criterion for positivity and not the proportion of the stained cells [21]. In some studies, the criteria for positivity were not described. The issue of intraobserver and interobserver variability was seldom addressed.

Importantly, the criteria of the case selection were generally discussed only briefly. Often the criteria for determining whether a tumour was primary or metastatic were not stated or 'the clinical data' or 'the patients files' were taken as the ultimate proof. In several studies, 'histologically-proven cases' were analysed. Only two autopsy-based studies were found that addressed the criteria of case selection [4,20]. Very often the tumours were not histologically-typed and subtyped, and intratumoral heterogeneity was not addressed; this is an obvious weakness, indicating the risk of phenotyping an erroneously categorised tumour and considering the phenotype to be typical for this category.

The difficulties of correlating the results of the analysed studies are obvious, but not unique, for the field of cytokeratin immunophenotyping. In practice, in the absence of rigorous and generally accepted international standards, variations of the criteria used in published studies must be accepted. However, I found that adjusting the results to a common system of evaluation is almost impossible. Thus, the tables in the present review contain cases considered positive if they were considered so in the original articles.

Although these differences can be considered to be highly significant, the generated data seem to be coherent and can be useful if:

1. sufficient data are collected about the stability of CK20 and CK7 expression in the primary tumour and its metastases,
2. sufficient data are collected about the frequency of CK20 and CK7 expression in primary adenocarcinomas (and other tumours), including the primary tumours of the organs that are the most usual sites of metastases,
3. proper clinical information, a simple immunohistochemical algorithm, and a probabilistic approach are used.

3. Stability of CK20 and CK7 expression

The successful diagnostic use of the expression of a certain antigen by tumour cells depends on the stability of the expression, despite disease progression. The diagnostic use can be compromised if the tumour cells lose or gain expression of the antigen during dedifferentiation or metastatic spread. Table 1 shows the percentages of primary and metastatic adenocarcinomas that stained positively for CK20 in the cited references. It is easy to conclude that adenocarcinomas of the reported organs differ significantly in the stability of the expression of CK20. Colorectal carcinomas are the best example of tumours that retain their CK20 expression during metastases. Ductal and lobular breast carcinomas, urothelial tumours (mentioned despite the fact that they are not adenocarcinomas), and endometrial, prostatic and kidney adenocarcinomas also seem to be stable. In my experience, CK20 is a very stable antigen

Table 1
Percentage of CK20+ cases in reported primary and metastatic adenocarcinomas

	All	Primary	Metastatic	*P* value	References
Colon	92% (394/430)	91% (177/194)	93% (217/233)	0.5	[2,5,6,7,10–12][a] [2–6,8,9,11–14,19,23][b]
Stomach	54% (93/172)	71% (32/45)	48% (61/127)	0.01	[2,5,10][a] [2–5,8,15][b]
Biliary	51% (57/111)	55% (48/87)	38% (9/24)	0.1	[5,22,23][a] [3–5][b]
Pancreas	44% (65/148)	44% (34/77)	44% (31/71)	0.8	[5,10,22][a] [3–5,8][b]
Breast, ductal	8% (38/488)	7% (18/248)	8% (20/240)	0.8	[2,5,10,11,16][a] [2–5,8,11,14,15,17][b]
Breast, lobular	5% (8/158)	6% (7/123)	3% (1/35)	0.7	[5,10,18][a] [5,8,18,24][b]
Endometrium	18% (14/80)	19% (12/64)	13% (2/16)	0.7	[2,5,10,11][a] [2,3,5,11,14][b]
Ovarium, non-mucinous	19% (73/382)	25% (62/246)	8% (11/136)	0.0005	[2,5,7,9,10,12,13][a] [3–5,8,12,15][b]
Lung	8% (35/426)	9% (26/282)	20% (29/144)	0.004	[5,6,10,11,17,19,20][a] [3,5,6,8,14,15,19][b]
Urothelium	78% (60/77)	78% (49/63)	79% (11/14)	0.7	[5,10,21][a] [3,5][b]
Prostate	21% (23/109)	19% (15/77)	25% (8/32)	0.6	[5,21][a] [3–5,14,21][b]
Kidney	4% (5/122)	7% (5/69)	0% (0/53)	0.1	[4,5,11][a] [3,5,8,11,14][b]

[a] These are references for primary tumours.
[b] These are references for metastases.

in certain types of breast carcinoma and their metastases. None of 10 metastatic medullary carcinomas and only one of 21 metastatic lobular carcinomas of the breast differed in CK20 positivity from their primary carcinomas [24,33]. However, lung, ovarian and stomach adenocarcinomas showed statistically significant differences in CK20 expression when the primary and metastatic locations were compared.

Table 2 contains comparable data about CK7 expression. In addition to the differences in expression of this antigen in the primary compared with metastatic lung and non-mucinous ovarian carcinomas, the same kind of differences occurred in ductal breast carcinomas.

To explain these differences, the data in Tables 1 and 2 are totals of the reported primary and metastatic cases and not necessarily a comparison of the phenotype of the primary and metastatic tumours in the same patient. The numbers of the reported cases in different categories also vary considerably. These differences once more highlight the importance of the criteria of case selection for this type of study.

4. CK20 expression in the primary tumours of the organs representing the most usual sites of metastases

Blood and bone marrow do not normally contain CK20. Detection of CK20 by polymerase chain reaction (PCR) technique or immunohistochemistry in these tissues is consistent with tumour spread. However, the clinical relevance of circulating tumour cells and 'minimal disease' in the bone marrow is not well defined.

Adenocarcinoma structures in bones (bone marrow) and brain are always consistent with metastasis. Searching for the unknown primary tumour in these cases should include CK20 phenotyping, however, only approximatley one-third of all adenocarcinomas stain positively for this marker. CK20 positivity has been reported in 4% of cases of glioblastoma multiforme [14].

Absence of strong and diffuse CK20 expression in mesothelium and mesotheliomas [2,5,10] is important information because of the well-known differential diagnostic difficulties in discriminating reactive and neoplastic mesothelial cells from cells of metastatic adenocarcinomas. In my opinion, detection of CK20-positive cells in cytological material (serous fluids) or histological biopsies from serous membranes favours the diagnosis of metastatic carcinoma (adenocarcinoma, urothelial carcinoma, squamous carcinoma, or Merkel cell carcinoma). Nevertheless, only approximately one-third of metastatic adenocarcinomas can be detected by CK20, and CK20 negativity does not exclude metastasis. One study [34] reported CK20 positivity in a large number of malignant epithelioid pleural mesotheliomas. The otherwise well-designed study was carried out in cases 'confirmed by clinicopathological evaluation' and 'without a minimum cut-off for the determination of positivity.' In my experience, weak focal positivity can appear in the mesothelial cells of an inflammatory exudate, and focal CK20 positivity (less than 10% of tumour cells) may appear in some cases (3/14) of epithelial malignant mesothelioma (autopsy-verified cases) [20]. Despite that, in my opinion, CK20 must be included in the panel of other well-established markers used routinely to differentiate mesothelial versus epithelial cells, because a strong and diffuse positive reaction indicates metastasis.

Only 9% of primary lung adenocarcinomas are CK20-positive, which means that a CK20-positive adenocarcinoma in the lung is more probably metastatic than primary. Considering primary liver adenocarcinomas, hepatocellular carcinomas are delineated as CK20-positive in only 7% (2/30 reported cases) [10] and cholangiocellular carcinomas as CK20-positive in 51% of

Table 2
Percentage of CK7+ cases in reported primary and metastatic adenocarcinomas

	All	Primary	Metastatic	*P* value	References
Colon	16% (61/389)	16% (37/225)	15% (24/162)	0.9	[2,6,7,10–12,25,26][a] [2,4,6,9,11–14,19,23,25,26][b]
Stomach	51% (55/107)	55% (30/55)	42% (22/52)	0.2	[2,10,25,26][a] [2,4,15,26][b]
Biliary	91% (95/104)	90% (78/87)	100% (17/17)	0.3	[22,23][a] [4][b]
Pancreas	95% (74/78)	98% (54/55)	87% (20/23)	0.16	[10,22,25,26][a] [4][b]
Breast, Ductal	86% (271/314)	90% (192/213)	78% (79/101)	0.007	[2,10,11,16,25,27–2][a] [2,4,11,14,15,17][b]
Breast, Lobular	95% (115/121)	94% (94/100)	100% (21/21)	0.5	[10,18,29][a] [18][b]
Endometrium	95% (60/63)	96% (49/51)	92% (11/12)	0.5	[2,10,11,25][a] [2,11,14,24][b]
Ovarium, Non-mucinous	83% (287/344)	88% (238/272)	68% (49/72)	0.0005	[2,7,9,10,12,13,25,26][a] [4,12,15,26]
Lung	96% (289/302)	98% (238/242)	73% (51/70)	0.0005	[6,10,11,17,19,20,30][a] [6,14,15,19][b]
Urothelium	95% (101/106)	95% (101/106)			[10,21,26][a]
Prostate	12% (13/112)	11% (9/82)	13% (4/30)	0.9	[10,21,25,26][a] [4,14,21][b]
Kidney	14% (11/79)	17% (11/66)	(0/13)	0.2	[10,11,25,26][a] [11,14]

[a] These are references for primary tumours.
[b] These are references for metastases.

cases. While diagnosing a primary liver tumour in cases of hepatocellular carcinoma is relatively easy (on the basis of histology and the useful canalicular positivity for polyclonal carcinoembryonic antigen (CEA)), delineating a primary cholangiocellular tumour and a metastatic adenocarcinoma in the liver is often impossible based on histology and CK20 expression.

Primary ovarian adenocarcinomas also express CK20. Approximately 25% (62/246) of non-mucinous tumours (endometrioid cancers included) (Table 1) and up to 67% (31/46) of mucinous tumours contain CK20 [2,5,10,12,13]. The histological picture and CK20 expression in these cases, especially in endometrial adenocarcinomas, are of limited value in discriminating primary and secondary ovarian tumours.

However, adding CK7 to CK20 in a simple immunohistochemical algorithm provides a new perspective. As shown in Table 3, lung adenocarcinomas are almost always CK7-positive. Most of the reported cases of primary mucinous and non-mucinous ovarian carcinomas, as well as all primary cholangiocellular carcinomas in the reviewed literature stating the CK20/CK7 phenotype of the tumours, were CK7-positive. This means that CK7 negativity can be used as a marker of the metastatic character of the adenocarcinomas in the lungs, liver and ovaries. Table 2 also confirms the high prevalence of CK7 positivity in primary lung, ovarian and biliary adenocarcinomas; however, this also indicates that ductal breast carcinomas, lung adenocarcinomas, and non-mucinous ovarian carcinomas may lose CK7 expression when they metastasise.

5. CK20/CK7 phenotype of the most frequent primary and metastatic adenocarcinomas

Analysis of the origin of a metastatic adenocarcinoma should include two algorithmic steps. The first step discriminates between the metastases and the primary adenocarcinomas of the target organ. The second step, in cases of metastases of uncertain origin, directs the clinicians' focus to the most probable primary site. The phenotypic distribution of the cases with reported CK20/CK7 expression in the reviewed literature is presented in Table 3. The value of this phenotype in diagnostic pathology is discussed elsewhere [2–4,6,7,35]. In this review, only three practical basic principles are discussed.

Use of a detailed database is essential for interpreting tumour phenotyping. After considering the difficulties in comparing the different studies, an assembled database should not be viewed as the absolute truth, but rather as a collection of existing information. Furthermore, different tumours express an antigen in a certain percentage of cases, which is only rarely 100%; Merkel cell carcinoma is a good example [5]. This percentage of antigen expression is more informative than a simple general statement or using + or + + +. For example, stating that colorectal carcinoma shows the CK20+/7- phenotype in 78% of the cases is more informative than a statement that colorectal carcinomas are usually CK20+ and CK7−. In my opinion, this 'probabilistic' approach is the appropriate way of informing clinicians of the probability of finding the primary tumour in the proposed site.

Clinical information is also essential. Knowledge of a previously diagnosed primary tumour or the location of suspicious lesions (e.g. pleura or a pelvic mass) directs the attention of the pathologist and may indicate inclusion of additional markers in phenotyping. The pathologist has to become an active member of the clinical team solving a well-defined problem. Well-formulated clinical questions are the basis for rational and successful use of immunohistochemistry. Good examples are the use of CK20 and CK7 for distinguishing metastatic lobular carcinomas from gastrointestinal signet ring cell carcinomas [24] or in distinguishing urothelial from prostatic carcinoma [21].

In daily diagnostic work, simple algorithms with only a few markers included should be used. More complicated algorithms, although possibly more accurate, can be expensive and difficult to interpret. In the search for

Table 3
Distribution of CK20/CK7 phenotype in adenocarcinomas of different organs (from Refs. [4,6,9,10,12,13,17,21])

Primary + metastatic	20+/7−	20+/7+	20−/7+	20−/7−	Total
Colon	78% (161)	12% (25)	1% (2)	9% (18)	100% (206)
Stomach	33% (13)	33% (13)	24% (9)	10% (4)	100% (39)
Biliary		24% (4)	76% (13)		100% (17)
Pancreas	7% (3)	48% (22)	41% (19)	4% (2)	100% (46)
Lung		8% (13)	84% (126)	8% (13)	100% (152)
Breast	1% (1)	11% (7)	88% (57)		100% (65)
Ovary, non-mucinous		7% (3)	93% (39)		100% (42)
Ovary, mucinous		76% (13)	14% (4)		100% (17)
Prostate	14% (11)	1% (1)	9% (7)	76% (62)	100% (81)
Urothelial	4% (1)	61% (17)	21% (6)	14% (4)	100% (28)
Total	190	118	282	103	693

an unknown primary tumour, staining should begin with CK20. As previously mentioned, CK20 positivity alone is a sign of metastasis in certain locations. The CK20+ group of tumours then can be subdivided according to CK7 negativity or positivity. CK7 negativity alone is a highly probable sign of metastasis of tumours of the ovaries, liver and lungs. The CK20- tumour group cannot be meaningfully subdivided by CK7. Antibodies against oestrogen receptors and prostate-specific antigen (PSA) must be added to the antigen panel used in this group.

6. Conclusions

Although there are significant differences in technical background, case selection and interpretation of the results, this review of 29 studies showed the practical value of determining CK20 and CK7 in adenocarcinomas. Detection of CK20 in blood, bones (marrow), brain, serous membranes and fluids indicates metastatic tumour spread. The CK20+/7− phenotype indicates metastatic adenocarcinoma, most often from the colon or rectum, not only in bones, brain, or serous membranes, but also in liver, ovaries and lungs. The CK20−/7− phenotype indicates metastatic adenocarcinoma, most often of the prostate, in all the previously mentioned sites.

Determining the CK20/CK7 phenotype of the tumour also can be useful in certain clinical situations, such as discriminating prostate and urothelial carcinomas. An algorithmic and probabilistic approach and use of a detailed database are recommended for effective assessment.

The collection of additional cases with clearly identified primary location, tumour type, and tumour subtype is necessary to prove the results of these studies and to extend CK phenotyping to rare tumour types. High-quality immunostaining, preferably with automation and external quality control has to be used. Rigorous international standards are needed in the technical assessment and interpretation of the immunohistochemical staining. Studies comparing the phenotypes of the metastases to those of the primary tumours are necessary to assess the stability of antigen expression. Finally, the challenging problem of intratumoral heterogeneity also has to be addressed.

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