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Original Article

Cytokeratin 7 and cytokeratin 20 expression in colorectal adenocarcinomas

Reyhan Bayrak*, Sibel Yenidünya, Hacer Haltas

Department of Pathology, School of Medicine, Fatih University, 06540 Emek, Ankara, Turkey

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ABSTRACT

Cytokeratin 7 (CK7) and cytokeratin 20 (CK20) are low molecular weight cytokeratins. The expressions of CK7 and CK20 have been studied in various primary and metastatic carcinomas. Their expression patterns may help to distinguish the site of origin of metastatic carcinomas. We investigated the expressions of CK7 and CK20 in 196 cases of colorectal carcinoma. Paraffin sections of 196 colonic adenocarcinomas were randomly selected, retrieved, and immunostained for CK7 and CK20 with a standard avidin–biotin complex method. CK7 was expressed in 34/196 (17.3%) and CK20 in 159/196 (81.1) cases of colorectal adenocarcinoma. CK7−/CK20+ had the greatest proportion (65.8%) in colorectal carcinomas. The CK7+/CK20+ immunophenotype was identified in 30/196 (15.3%), CK7−/CK20− in 33/196 (16.9%), and CK7+/CK20− in 4/196 (2%) colon adenocarcinomas. The CK7 and CK20 expression patterns were different in colorectal carcinomas according to histological grade, location of the tumor, and lymph node metastasis. CK20 positivity was more common in low grade carcinomas than in high grade carcinomas (85.1% versus 47.6%) and in rectal and sigmoid carcinomas than in proximal colon carcinomas (88.2% versus 63.2% and 88.9% versus 63.2%, respectively). Furthermore, CK7 expression was more common in tumors with lymph node metastasis than in non-metastatic tumors (25.3% versus 11%). In conclusion, a considerable number of colorectal carcinomas showed reactivity to CK7 (17.3%) or no reactivity to CK20 (18.9%). Therefore, CK7 positivity or CK20 negativity does not rule out a colorectal origin of metastatic carcinoma.

Introduction

A metastatic lesion can be the first clinical presentation of a neoplastic process, probably accounting for more than 10% of all new tumor diagnoses [1]. Although modern imaging technology has resulted in improvements in the identification of primary tumors, in most patients, the origin remains unknown. Therefore, the pathologist has acquired an increasingly important diagnostic role in characterizing the site of origin of these tumors [2].

Immunohistochemistry has been shown to be a useful tool in the identification of the primary site. It is the least invasive diagnostic technique and is certainly less expensive than imaging techniques. The generation and availability of new antibodies that are specific for a wide range of antigens and tissues that work on routinely fixed, paraffin-embedded material will ensure that this technique will continue to offer useful information in the search for elusive primary sites.

Cytokeratins are among a group of approximately 20 cytoskeletal structural proteins present in normal epithelia, and their expression is maintained during malignant transformation [3]. Because CK expression is usually preserved in neoplastic cells, the use of specific antibodies is of value in determining the origin of metastatic carcinomas. In normal tissue, CK7 is expressed in simple epithelia of breast, lung, mesothelium, female genital tract, and urinary bladder, with limited expression in gastric and intestinal mucosa [4,5]. CK20 is a major cellular protein of mature enterocytes and goblet cells in the intestinal tract, urothelium, and Merkel cells in the skin [6]. The relative expression of CK20 and CK7 has been observed to vary among different epithelial tumors, and these markers are currently used as diagnostic tools to help determine the site of origin of metastatic carcinomas [7–11]. For example, the CK20+/CK7− immunoprofile has been considered characteristic for colonic adenocarcinoma, and has been used to distinguish it from adenocarcinomas of other origins, such as female genital tract, lung, breast, and liver. Because cytoplasmic CK7 expression in colorectal adenocarcinoma is considered rare, CK7 positivity in metastatic carcinoma is often considered to exclude a colorectal primary [9,12,13]. However, not all colorectal carcinomas show the CK20+/CK7− expression pattern; a substantial proportion of colorectal carcinomas are CK20− or CK7+ [14–16].

In the present study, we investigated the expression profile of CK7 and CK20 in 196 primary colorectal adenocarcinomas in the light of the potential applicability of these markers in the clinical context of metastatic adenocarcinomas.

* Corresponding author at: Fatih Üniversitesi Tıp Fakültesi, Alparslan Türkeş Cad. No. 57, 06510 Emek, Ankara, Turkey. Tel.: +90 3122035584; fax: +90 3122035460.
E-mail address: bayrakreyhan@yahoo.com (R. Bayrak).

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Materials and methods

Case selection and tissue samples

Paraffin-embedded tissue sections were obtained from archival material from 196 patients who underwent resection for primary colorectal carcinoma between January 2005 and December 2009. All cases were selected from patient files at the Department of Pathology, Fatih University Hospital. Pathological findings, including histological type, histological differentiation, depth of invasion, and lymph node status, were obtained from hematoxylin and eosin-stained sections.

All cases were reviewed to confirm the diagnosis. WHO criteria were used for histological typing [17]. Postoperative pathological staging was performed according to the American Joint Committee on Cancer (AJCC) TNM staging system [18]. One paraffin block with the maximum amount of tumor and proper fixation was selected from each case for immunohistochemical (IHC) studies.

Among the 196 cases of colorectal carcinoma, 174 were classified as adenocarcinoma, 14 as mucinous carcinoma, and 8 as signet-ring cell carcinoma. Eight cases were categorized as T1, 16 as T2, 148 as T3, and 24 as T4 according to the American Joint Committee on Cancer staging system. Based on histological grading, the carcinomas were classified as 175 low-grade carcinomas (well-moderately differentiated) and 21 high-grade carcinomas (poorly differentiated and undifferentiated carcinomas). According to their location, tumors were classified as rectal carcinomas ($n = 85$), sigmoid carcinomas ($n = 54$), and carcinomas proximal to the rectosigmoid region ($n = 57$). Lymph node metastasis was observed in 87 patients.

Immunohistochemistry

Four-μm-thick sections were cut from blocks of paraffin-embedded tissue, deparaffinized, and rehydrated as usual. To reduce non-specific background staining due to endogenous peroxidase, slides were incubated in Hydrogen Peroxide Block for 15 min. Before immunostaining, antigen retrieval was performed by incubating the slides for 15 min with pepsin (LabVision; catalog no. AP-9007) at a concentration of 1 mg/ml for CK20. Slides were microwaved in 10 mM of citric acid at pH 6.0 for 20 min for CK7. The slides were incubated for 60 min with primary antibodies to CK7 (clone OV-TL 12/30, LabVision/NeoMarkers; 1:50) and CK20 (clone Ks 20.8, Dako; 1:50) at room temperature. The standard avidin–biotin–peroxidase complex (ABC) technique was performed using the LabVision Secondary Detection Kit (UltraVision Detection System Anti-polyvalent, HRP). AEC and DAB were used as chromogen. All slides were counterstained with Mayer's hematoxylin.

Positive immunostaining for CK7 and CK20 was identified in the cytoplasm, cell membrane, or both tumor cell components. The immunostaining result was assessed semiquantitatively. The percentage of positive cells was recorded as follows: less than 5%, 5–25%, 26–50%, 51–75%, and more than 75%. Cases that stained less than 5% were considered negative, and all others as positive for statistical purpose. Normal colonic mucosal tissue was used as a CK20-positive control, and breast carcinoma tissue was used as a CK7-positive control. We used the normal colorectal tissue of resected specimens to evaluate the normal expression patterns of CK7 and CK20.

Statistical analysis

The results were evaluated using Pearson's chi-square test. p values less than 0.05 were considered significant. SPSS 13.0 for Windows was used for all statistical analyses.

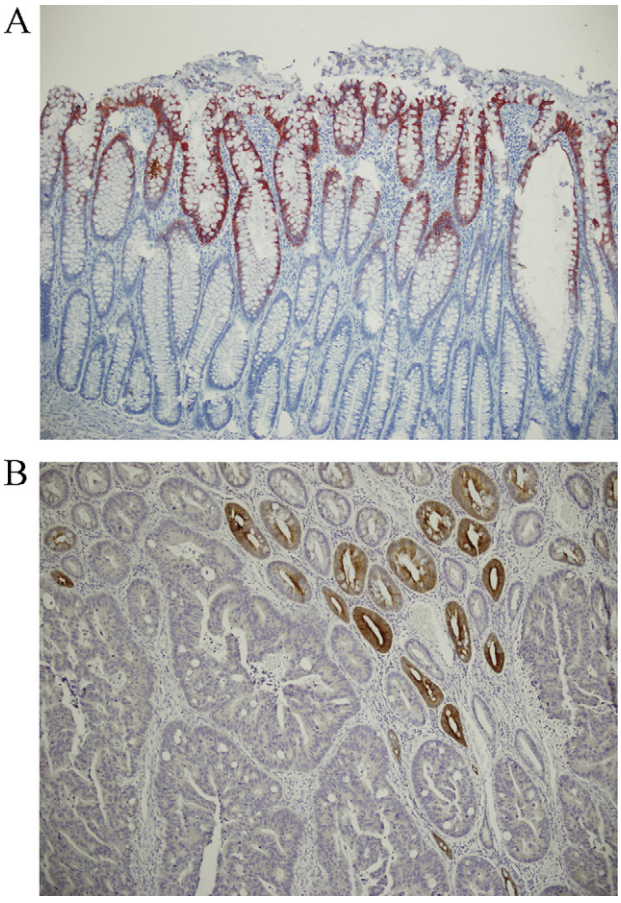

Fig. 1. Normal colonic epithelium with cytokeratin 20 (A) and cytokeratin 7 (B) immunoreactivity (*Note*: The lack of CK7 immunoreactivity in neoplastic gland) (100×).

Results

Histologically normal colonic epithelium stained positively for CK20. Cytoplasmic CK20 immunoreactivity was more prominent in the surface epithelium and tended to diminish toward the crypts (Fig. 1A). We also observed CK7 immunoreactivity in normal colonic epithelium, and this immunoreactivity tended to be focal in the surface and the crypt epithelium (Fig. 1B).

CK20 expression was detected in 159 of 196 (81.1%), and CK7 expression was detected in 34 of 196 (17.3%) colorectal carcinomas. CK20 reactivity was diffuse in the majority of colon adenocarcinomas (more than 50% of cells were positive) in 54% of the cases (Fig. 2). Among the 34 cases of colorectal carcinoma expressing CK7, 22 were focally positive, and 12 were diffusely positive (Fig. 3). In diffusely CK7-positive cases, 4 were CK20-negative (Fig. 4). The results are summarized in Table 1.

In terms of the combined expression of CK7 and CK20, the proportion of CK7−/CK20+ was highest, accounting for 65.8% (129 of 196) of colorectal carcinomas. The CK7+/CK20+ immunophenotype was expressed in 30 (15.3%), and CK7−/CK20− in 33 (16.9%) cases. The CK7+/CK20− expression pattern was observed in only 2% (4 of 196) of colorectal carcinomas (Table 2).

CK7 and CK20 expression in colorectal carcinoma was compared with the clinicopathological characteristics (Table 3). No association between CK7 expression and anatomical location of carcinomas, tumor type, tumor stage (pT), and grade was found. There was a correlation between CK7 expression and lymph node metastasis, as CK7 expression was more common in tumors with lymph node metastasis than in non-metastatic tumors (25.3% versus