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Original article

Aberrant pattern of the cytokeratin 7/cytokeratin 20 immunophenotype in colorectal adenocarcinomas with BRAF mutations

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ABSTRACT

Cytokeratin 7 (CK7) and 20 (CK20) are used for the differential diagnosis of metastases from colorectal carcinomas (CRC), which are usually CK7−/CK20+, and other tumors. In our study, we performed immunohistochemical staining with CK7 and CK20 in 52 randomly selected cases of CRC and analyzed microsatellite instability status and BRAF mutations to identify those factors that may determine the changing pattern of CK7/CK20 immunophenotype in these tumors. CK7 was negative in all microsatellite stable tumors (MSS), but all carcinomas presenting microsatellite instability (MSI) and BRAF mutations were diffusely positive for this marker. CK20 was diffusely expressed in 79.06% of MSS tumors. Regarding MSI, in case with no BRAF mutations, a progressive decrease in CK20 expression was noted, and in BRAF-mutated adenocarcinomas, no expression of CK20 was observed. It seems that in case of MSI located on the proximal colon, which also presents BRAF mutations, CK20/CK7 may present a changing immunophenotype pattern, which may complicate the differential diagnosis of metastatic tumors. This is the first reported study of the relationship between CK20/CK7 immunophenotype, BRAF mutations and microsatellite status in CRC.

Introduction

Bayrak et al. [1] recently suggested that 17.3% of colorectal adenocarcinomas (CRC) expressed cytokeratin 7 (CK7) and 18.9% of cases did not express cytokeratin 20 (CK20), although most authors admit that CK20 is of great importance in the differentiation of colonic metastases from other metastatic tumors like lung, bladder, breast carcinomas and cholangiocarcinomas [4,5,8].

On the one hand, CK7 is usually expressed in the normal epithelia of the lung, mesothelium, urinary bladder and female genital tract, and, rarely, it may be observed in gastric and intestinal normal glands, but most authors believe that it is not found in the normal colonic mucosa [9,10]. On the other hand, CK20 specifically stains the normal gland cells of the colonic mucosa and Merkel cells and, more rarely, its expression may be seen in the urothelium or other mucosas [10,14].

Based on these characteristics, the immunophenotype CK7/CK20 is used in daily diagnoses to differentiate CK20 expresssing metastases of colorectal adenocarcinomas (CRC) from lung, ovarian or bladder carcinomas, which are usually stained with CK7 [2,12].

In our study, we analyzed the immunohistochemical (IHC) expression of both CK7 and CK20 in CRC to emphasize the real importance of these antibodies in the differential diagnosis of metastatic adenocarcinomas and to identify those factors which may induce the changing pattern of CK7/CK20 immunophenotype in these tumors. We also tried to correlate the expression of these markers with the microsatellite status and BRAF mutations. These aspects have not been mentioned in the English literature yet.

Material and methods

In 52 randomly selected cases of colorectal adenocarcinoma (26 from proximal and 26 from distal colon and rectum), we performed immunohistochemical (IHC) staining with CK7 and CK20 and molecular examinations to determine BRAF mutations and microsatellite status.

UltraVision system by D-Line, LabVision (Fremont, CA, USA), was used for immunohistochemical reactions in formalin-fixed, paraffin-embedded tissues. CK7 (clone OV-TL 12/30) and CK20 (clone Q6) antibodies were also provided by D-Line, LabVision. Sections were deparaffinized, incubated at 100 °C with pepsin and washed with distilled water previous to hydrogen peroxide incubation. Subsequently, they were washed with Tris buffered saline (TBS) and incubated with primary antibodies for 60 min. Then, they were washed with TBS and covered with streptavidin peroxidase solution for 5 min. After this, they were washed with TBS

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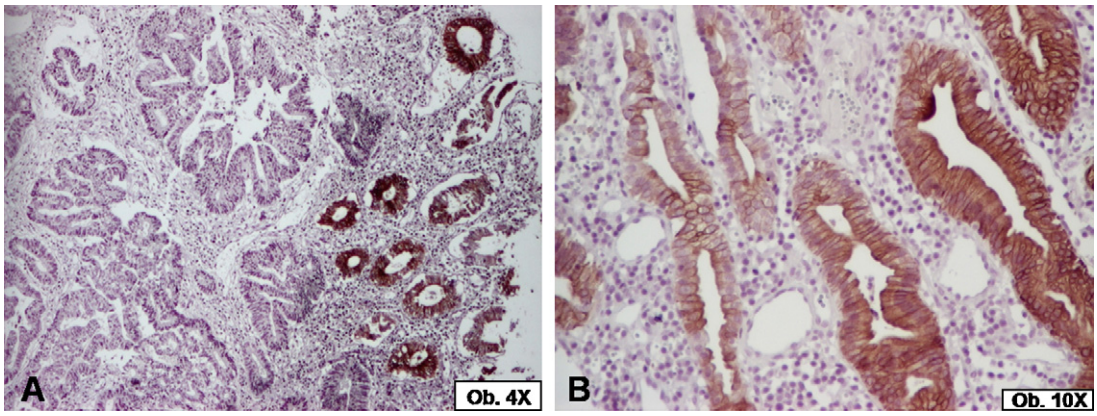

Fig. 1. A microsatellite stable colon adenocarcinoma without BRAF mutations, with cytokeratin 7 immunoreactivity in normal mucosa (A). The tumor cells present diffuse expression of cytokeratin 20 (B) and no staining for cytokeratin 7 (A).

and covered with biotinylated goat anti-polyvalent solution for further 5 min. The development was performed with dyaminodihydrochlorid benzidine solution (DAB) for 3–5 min. Counterstaining was performed with Mayer's hematoxylin.

Both CK7 and CK20 expressions were evaluated in the cell membrane and also in the cytoplasm. We considered those cases negative which expressed these markers in less than 5% of the cells. Focal positivity was considered as the presence of antibody expression in 5–40% of the tumor cells, the other cases were considered to have diffuse positivity.

To determine microsatellite instability, DNA was extracted from formalin-fixed, paraffin-embedded tissues. We used real-time PCR (Roche GmbH, Mannheim, Germany), the method of melting point analysis, as well as microsatellite mononucleotide markers BAT25 and BAT26 by Roche. Exon 15 BRAF mutations were determined using real-time PCR.

Results

Forty-three of the 52 cases were microsatellite stable carcinomas (MSS), and 9 presented microsatellite instability (MSI), all 9 being located on the proximal colon. Four of the 52 cases presented BRAF mutations. All four cases were also MSI carcinomas.

Regarding the normal mucosa, independent of the microsatellite status, BRAF mutations or other factors, CK20 was positive in 41 of the 52 cases (78.84%), the number of stained cells being higher in the surface epithelium. CK7 was focally positive in the normal mucosa in 10 of the 52 carcinomas (19.23%), its intensity being higher in the crypt epithelium (Fig. 1).

We found some differences between MSI and MSS cases regarding the CK7/CK20 immunophenotype in tumor areas. In 34 of the 43 MSS cases (79.06%), the tumor cells were diffusely positive for CK20, but no CK7 expression was observed in these tumors (Fig. 1). In all 9 MSI cases, CK20 was either focally expressed or negative, independent of the other clinicopathological parameters (Table 1). No MSI cases with diffuse positivity were identified for CK20. The CK7 expression was dependent on BRAF mutations and lymph node status. It was diffusely positive in all MSI cases with BRAF mutations and negative in all carcinomas without BRAF mutations (Figs. 2 and 3), except one case. In this MSI case (case 5), which had no BRAF mutations but lymph node metastases, CK7 was focally expressed.

Discussion

According to other authors, the immunophenotype CK7−/CK20+ is characteristic of colorectal adenocarcinomas [2,12,17].

Few recent studies have revealed that this immunophenotype may present some changes. During carcinogenesis, some dysplastic glands were CK7-positive, but the normal mucosa was CK7−/CK20+ [9,14]. In colorectal adenocarcinomas, the superficial glands of the normal mucosa express CK20 and remain negative for CK7, but the crypts may present positivity for CK7 [1]. At the same time, both markers may have different patterns in the tumor cells. These cells may be either diffusely positive for CK20, or their reduced expression is obviously emphasized [6]. In some cases, CK7 may be expressed by the tumor cells, but the positivity rate ranges from 0 to 74% according to previously published studies [1,11,16].

Fewer than 10 published studies have analyzed the reasons for the changes in the immunophenotype pattern in CRC. In only one of these studies was microsatellite instability also considered [6]. These studies revealed that CK7 expression was more common in advanced high-grade carcinomas with lymph node metastases located on the proximal colon, but CK20 was expressed more frequently in carcinomas of the distal colon and rectum [1,3].

Table 1
The correlation between clinico-pathological parameters, CK7 and CK20 expression and BRAF mutations in colorectal carcinomas with microsatellite instability (BRAF-MUT = mutant; M = male; F = female).

No. of case	Age (years)	Gender	Microscopy	Tumor stage	BRAF status	CK7	CK20
1	55	M	Mucinous carcinoma	pT3N0	Wild type	Negative	Focally positive
2	75	M	High-grade adenocarcinoma	pT3N0	**MUT**	Diffuse positive	Negative
3	66	M	Mucinous carcinoma	pT3N0	**MUT**	Diffuse positive	Negative
4	56	F	Mucinous carcinoma	pT4N0	**MUT**	Diffuse positive	Negative
5	49	M	Mucinous carcinoma	pT3N2	Wild type	Focally positive	Focally positive
6	73	F	Signet ring cell carcinoma	pT4N0	Wild type	Negative	Focally positive
7	73	M	Signet ring cell carcinoma	pT3N0	Wild type	Negative	Focally positive
8	63	F	Mucinous carcinoma	pT3N0	Wild type	Negative	Negative
9	63	F	Low-grade adenocarcinoma	pT2N0	**MUT**	Diffuse positive	Focally positive