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Original Article

Immunohistochemical Expression of CK20, CK7, and CDX2 in Colorectal Carcinoma in Correlation with Pathomorphological Characteristics

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Abstract

Introduction: Colorectal carcinoma is the third most common cancer worldwide. The usual immunophenotype of colorectal adenocarcinoma is CDX2 positive, CK20 positive, and CK7 negative. Aberrant expression is reported in a variety of colorectal carcinomas but its relation to morphological variables and survival data is still unclear.

Aim: The aim of this study was to investigate the correlation between the aberrant immunostaining of colorectal carcinoma and different clinicopathological characteristics.

Materials and methods: Immunohistochemical expression of CK20, CK7, and CDX2 was evaluated in 71 cases of colorectal carcinoma. Statistical analysis was performed to identify correlations between the morphological characteristics and the immunoprofile of colorectal carcinoma.

Results: Positive cytoplasmic and/or membranous signal for CK20 was observed in 66.2% of colorectal carcinomas. CK7 positive immunostaining was seen in 7% of the cases. In terms of combined expression of CK20 and CK7, the proportion of immunoprofile CK20+/CK7− was the highest, accounting for 46 out of 71 colorectal carcinomas, followed by CK20−/CK7−, then CK20−/CK7+ and CK20+/CK7+. Concerning CDX2, the majority of colorectal carcinomas (87.3%) showed positive staining. Statistically significant correlation was established between CDX2 expression and histologic grade and depth of tumour invasion. Loss of CK20 positivity was associated with higher histologic grade. No association between CK7 expression and histopathologic features was established.

Conclusions: The results support the heterogeneity of colorectal cancer. Over 35% of the cases in this study showed deviations from the expected immunoprofile. This should be taken into consideration when diagnosing colorectal carcinoma in metastatic regions.

Keywords

CRC, IHC, CK20, CK7, CDX2

INTRODUCTION

Colorectal carcinoma (CRC) is the third most common cancer worldwide after lung and prostate cancers in men and lung and breast cancers in women. It is also the third leading cause of cancer-related death in both sexes.[1,2] In 2018, colorectal carcinoma estimated 6.1% of the newly diagnosed cancers worldwide according to Global Cancer Statistics.[1]

The likelihood of colorectal cancers diagnosis increases after the age of 40, and rises sharply after the age of 50. Men are at a slightly higher risk for colorectal cancer than women. Other risk factors include environmental factors, such as nutrition, obesity, physical inactivity, smoking and alcohol consumption, personal history of adenomatous polyps, personal history of inflammatory bowel disease, and inherited risk factors. Approximately 5% to 10% of colorectal cancers are a consequence of recognized hereditary conditions, such as familial adenomatous polyposis (FAP) and hereditary nonpolyposis colorectal cancer (HNPCC), also called Lynch syndrome.[2,3]

More than 90% of colorectal carcinomas are adenocarcinomas with its histologic variants – mucinous, signet ring cell, medullary, micropapillary, serrated, and cribriform comedo-type **(Fig. 1)**. Other rare types of colorectal carcinomas include neuroendocrine, squamous cell, adenosquamous, spindle cell, and undifferentiated carcinomas.[3]

The most widely used immunohistochemical markers for colorectal carcinoma are CDX2, CK20, and CK7. CDX2, which is considered as a specific marker of the intestinal epithelial cells, is positive in almost all well-differentiated CRCs, but around 10%–20% of poorly differentiated and dedifferentiated carcinomas can be weakly positive or negative for CDX2. Cytokeratins are a group of approximately 20 cytoskeletal proteins present in normal epithelial cells and their expression is usually preserved in the neoplastic cells as well. The characteristic immunoprofile of colorectal carcinoma is CK20+/CK7−/CDX2+ **(Fig. 1)**. However, some primary colorectal carcinomas show deviations from this pattern. According to the relevant literature, the CK20+/CK7− immunoprofile is expressed in about 75%–95% of CRC, while the rest of the cases show different profiles.[3,4]

In the era of personalized medicine, the role of a pathologist in diagnosing CRC has greatly expanded. In addition to histopathologic diagnosis, surgical pathologists should provide also further prognostic parameters.

AIM

The aim of this study was to estimate the correlations between the expression of the three immunohistochemical markers CDX2, CK20, and CK7, and different pathological and morphological characteristics of CRC and to analyse the diagnostic, prognostic, and predictive role of the different patterns of CK20/CK7 immunoexpression.

MATERIALS AND METHODS

Case selection and tissue sampling

Paraffin-embedded tissue sections were collected from archival material from 71 patients who underwent hemicolectomies due to colorectal cancer between January 2017 and December 2018. The materials were reviewed by two pathologists to confirm the diagnosis and to evaluate the histological type, differentiation, depth of invasion, and lymph node status. WHO criteria were used for histological typing. Postoperative pathologic staging was performed according to the American Joint Committee on Cancer (AJCC) TNM staging system.

Immunohistochemistry

For immunohistochemical analysis, 4-µm-thick sections were cut from blocks of paraffin-embedded tissue. The antibodies used for the experiment are as follows: anti-CK 20 (SP33, Ventana, Roche) Rabbit Monoclonal Primary Antibody, anti-CK 7 (SP52, Ventana, Roche) Rabbit Monoclonal Primary Antibody and nuclear protein CDX-2 (EPR2764Y, Ventana, Roche). An automatic immunostainer (Ventana BenchMark GX) was used following the manufacturer's protocols.

Interpretation of immunohistochemical staining

For interpretation of the immunohistochemical results, a scale from 0 to 3+ was used, depending on the intensity of cytoplasmic and/or membranous signals for CK7, CK20, and nuclear signals for CDX2 relatively.

- 0: there is no evidence of cytoplasmic/membranous or nuclear expression;
- 1+: there is weak cytoplasmic/membranous or nuclear expression in tumour cell, seen at high magnification ×20;
- 2+: there is intermediate cytoplasmic/membranous or nuclear expression in tumour cells, seen at low magnification ×10;
- 3+ – there is strong cytoplasmic/membranous or nuclear expression in tumour cells, seen at low magnification ×4.

For statistical purposes, the absent (0) and weak (1+) signals were considered negative, whilst intermediate (2+) and strong (3+) signals were considered positive.

Statistical analysis

SPSS v. 19 was used to analyse the data statistically in this study. A p value <0.05 was considered statistically significant. Descriptive statistics were used for analysis of the clinicopathological parameters of the patient's group. Fisher's exact test was used to analyse correlations between

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CDX2, CK20, and CK7 expression and tumour location, grade, depth of invasion, lymph nodes. The coefficient of correlation of Kendall's tau-b (T_b) was used as an alternative of the Fisher's exact test.

RESULTS

Clinicopathological parameters of the patients

Seventy-one patients were included in the study (38 men and 33 women). Their mean age was 65±11 years. The majority of tumours were of the conventional type adenocarcinomas with slight predominance for the left colon localization **(Fig. 1)**. The relevant data are summarized in **Table 1**.

CK20, CK7, and CDX2 immunostaining profile

Positive cytoplasmic and/or membranous signal for CK20 was observed in 66.2 % of colorectal carcinomas **(Fig. 1)**. CK7 positive immunostaining was seen in 7% of the cases. In terms of combined expression of CK20 and CK7, the proportion of immunoprofile CK20+/CK7− was the highest, accounting for 46 out of 71 colorectal carcinomas, followed by CK20−/CK7−, then CK20−/CK7+ and CK20+/CK7+, with respectively 20, 4, and 1 cases.

Relation between CDX2 and histopathologic parameters of colorectal carcinomas

Concerning CDX2, the majority of colorectal carcinomas (87.3%) showed positive staining.

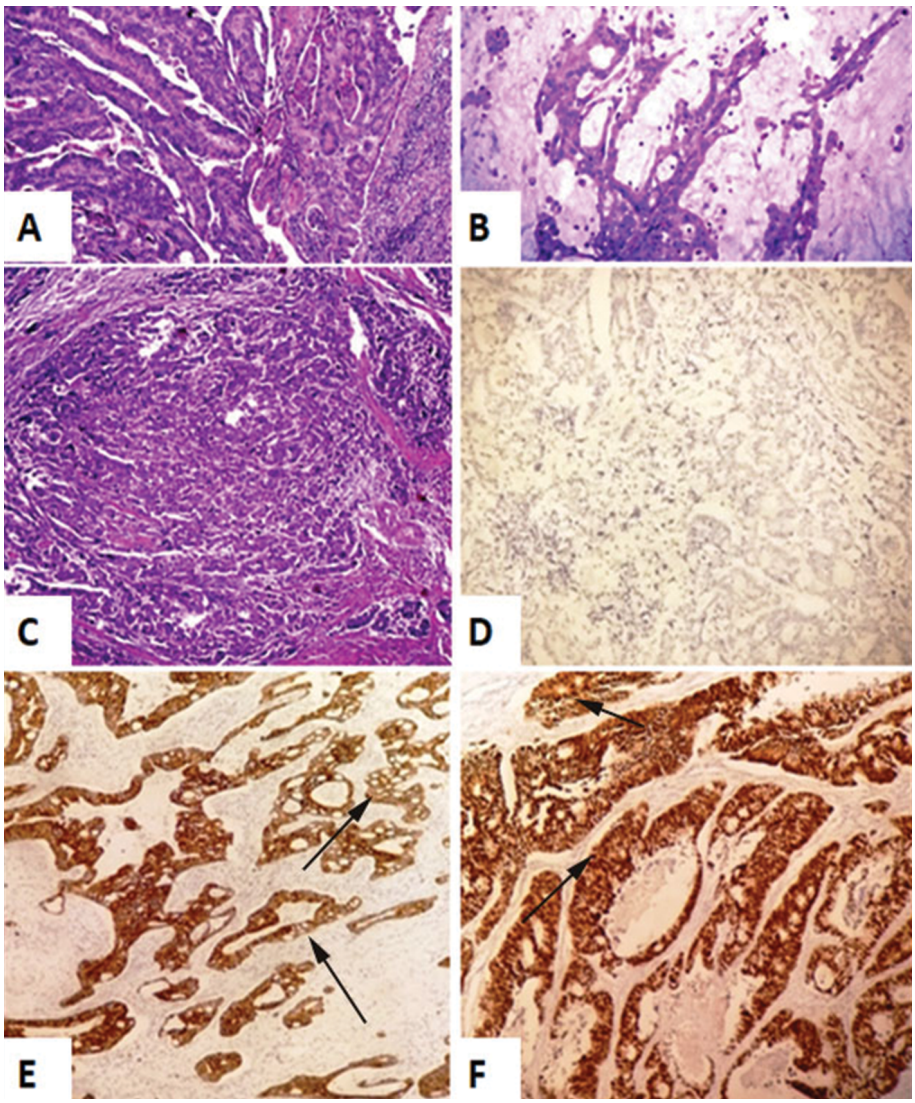

Figure 1. A. A conventional type of colorectal adenocarcinoma; **B.** Mucinous colorectal adenocarcinoma; **C.** Signet-ring cell colorectal adenocarcinoma; **D.** Negative CK7 immunostaining; **E.** Positive membranous signal for CK20, outlining the cell's contours (arrows); **F.** Positive nuclear staining for CDX2 (arrows).

Table 1. Clinicopathological parameters of patients (n=71)

Parameters	
Sex	
Male	38 (53.5%)
Female	33 (46.5%)
Age (years)	
Mean	65 years
Minimum	37 years
Maximum	83 years
Standard deviation	11 years
Tumour location	
Right colon	24 (33.8%)
Left colon	41 (57.7%)
Missing data	6 (8.5%)
Histologic type	
Conventional adenocarcinoma	64 (90.1%)
Mucinous adenocarcinoma	4 (5.6%)
Signet ring cell	1 (1.4%)
Medullary adenocarcinoma	1 (1.4%)
Papillary adenocarcinoma	1 (1.4%)
Grade	
Well-differentiated	8 (11.3%)
Moderately-differentiated	53 (74.6%)
Poorly-differentiated	10 (14.1%)
Primary tumour	
T1	0
T2	3 (4.2%)
T3	39 (54.9%)
T4	23 (32.4%)
Missing data	6 (8.5%)
Nodal metastasis	
Positive	50 (21.1%)
Negative	15 (70.4%)
Missing data	6 (8.5%)
Distant metastasis	
Positive	12 (16.9%)
Negative	24 (33.8%)
Missing data	35 (49.3%)

Detailed data concerning the correlation between CDX2 immunostatus and different histopathologic features of colorectal carcinomas are shown in **Table 2**. We used Fisher's exact test for non-parametric variables and then Kendall's tau-b correlation coefficient for confirmation of the results. A significant correlation was established between CDX2 expression and histologic grade of colorectal carcinomas (p=0.003; correlation coefficient = −0.328), and between CDX2 expression and depth of tumour invasion (p=0.006; correlation coefficient = −0.321). We observed no statistical significant difference between CDX2 immunostatus and tumour location and nodal metastases.

Relation between CK20 and histopathologic parameters of colorectal carcinoma

Table 3 shows the detailed data derived from the Fisher's exact test, concerning the correlation between CK20 immunostatus and different histopathologic features of CRC. However, significant correlation was found between CK20 expression and the histologic grade of colorectal carcinomas using the Kendall's tau-b correlation coefficient (T_b = −0.323, p=0.002). We observed no statistical significant difference between CK20 immunostatus and tumour location, depth of invasion, and nodal metastases.

Relation between CK7 and histopathologic parameters of colorectal carcinoma

We found no statistically significant correlations between CK7 immunoexpression and the different histopathological features.

DISCUSSION

Immunohistochemical methods are used on a daily basis for identification of the primary site of poorly differentiated metastatic tumours. In our study, we investigated the expression of three immunohistochemical markers, CDX2, CK20, and CK7, in association with different clinicopathological parameters in colorectal carcinomas. CDX2 is a transcription factor involved in the proliferation and differentiation of intestinal epithelial cells and its expression in adenocarcinomas of the gastrointestinal tract increases from esophagus to rectum. Cytokeratins are cytoplasmic intermediate filaments found in epithelial cells. They are distributed in a tissue-specific manner and usually carcinomas have a cytokeratin profile similar to that of the normal tissue of their origin. The two cytokeratin markers most commonly used for identification of the primary site of a tumour metastasis, are CK20 and CK7. CK7 is expressed in many ductal and glandular epithelia, such as these of the respiratory tract, mammary gland, endometrium, ovaries, biliary tract, apocrine and eccrine glands, urothelium and mesothelium. CK20 is specific for gastrointestinal tract epithelium, urothelium, and Merkel cells.[4]

Previous studies have reported the CDX2 positive expression in colorectal carcinomas to be around 90%, cytokeratin 20 positive expression – between 62% and 96% in different studies, and for cytokeratin 7 – up to 17%.[4-11] Our results are consistent with these results. It is noticeable that there is a wide variation in the percentage of CK20 positivity. This can be due to differences in the studied pop-

Table 2. Correlation between CDX2 immunostatus and clinicopathologic characteristics of colorectal carcinomas

Parameters	CDX2 retained	Loss of CDX2 expression	*P* value (Fisher's exact test)
Localization			1.00
Right colon	21 (88.1%)	3 (12.5%)	
Left colon	35 (85.4%)	6 (14.6%)	
Grade			0.028
Well-differentiated	8 (100%)	0 (0%)	
Moderately-differentiated	48 (90.6%)	5 (9.4%)	
Poorly-differentiated	6 (60%)	4 (40%)	
T category			0.013
T2	3 (100%)	0 (0%)	
T3	37 (94.9%)	2 (5.1%)	
T4	16 (69.5%)	7 (30.4%)	
Nodal metastases			0.672
Present	42 (84 %)	8 (16%)	
Absent	14 (93.3%)	1 (6.7%)	

Table 3. Correlation between CK20 immunostatus and clinicopathological characteristics of colorectal carcinomas

Parameters	CK20-retained	Loss of CK20 expression	*P* value (Fisher's exact test)
Localization			1.00
Right colon	16 (63.7%)	8 (36.3%)	
Left colon	27 (65.9%)	14 (34.1%)	
Grade			0.108
Well-differentiated	7 (87.5%)	1 (12.5%)	
Moderately-differentiated	36 (67.9%)	17 (32.1%)	
Poorly-differentiated	4 (40%)	6 (60%)	
T category			0.502
T2	1 (33.3%)	2 (66.6%)	
T3	26 (66.6%)	13 (33.3%)	
T4	16 (59.6%)	7 (30.4%)	
Nodal metastases			0.757
Present	34 (68 %)	16 (32%)	
Absent	9 (60%)	6 (40%)	

ulations, variations in immunohistochemical procedures, and interpretation criteria.

In terms of combined expression for CK20 and CK7, the most common immunoprofile is CK20+/CK7–, but up to 20% of the colorectal carcinomas may exhibit variations.[3] In our study, almost 65% of the cases presented with the typical profile. The second most common profile was CK20–/CK7– (28.2%), followed by CK20–/CK7+ (5.6%). Only one colorectal carcinoma had CK20+/CK7+ immunostatus. The results are similar to those reported by previously conducted studies.[5] It is worth noting that only four of the cases with an aberrant immunoprofile for CK20 and CK7 have lost their signal for CDX2. This finding emphasises the fact that CDX2 is the immunomarker with the highest sensitivity and specificity for colorectal carcinoma and reinforces pre-existing recommendations that it can be used alone for confirmation of the diagnosis of metastatic CRC, whilst CK20 and CK20 should always be used together in a panel.[3]

In this study, CDX2 staining was lost in 12% of colorectal carcinomas. It is still unclear what are the specific reasons associated with the decrease in expression for this marker. In a recent study, Olsen et al. indicated correlation between loss of CDX2 and tumour grade, stage, right-sided

tumour location, MMR-deficiency, CIMP-high, and BRAF mutations.[6] Lugli et al. found relationship between the loss of CDX2 expression and higher T stage, N stage, tumour grade, and proximal location in CRCs.[7] Bae et al. evaluated the expression of CDX2 in 713 cases of CRCs and established association between downregulation in CDX2 expression and proximal location, infiltrative growth, advanced T, N, M stages, and poor differentiation. They also stated that patients with loss of CDX2 showed worse overall survival.[8] In our cases, we estimated a weak downhill relationship between CDX2 expression and histologic grade (p=0.003, Kendall's tau-b correlation coefficient = −0.328) and T pathologic stage (p=0.006, Kendall's tau-b correlation coefficient = −0.321). We did not find statistically significant correlation with proximal location, N and M pathologic stages.

For CK7, 7% of the colorectal carcinomas included in our study showed positive staining. We estimated no statistically significant relationships between CK7 positive expression in CRCs and any clinicopathological features. However, in a recent study Fei et al. demonstrated that CK7 positive tumours were more likely to have strong metastatic and invasive features. The researchers found association of CK7 expression and location, differentiation, tumour stage, and lymph nodes metastases. It is interesting to note that CK7 positive cells were mainly distributed at the edge of cancer nests, at the invasion front, in tumour buds, in intravascular tumour emboli, and in tumours with micropapillary pattern.[9]

Our findings for CK20 positivity (66.2%) are in agreement with some previous studies. Bayrak et al. have described statistically highly significant relation between CK20 expression and anatomical site of the tumour process.[10] Park et al. observed an association between CK20 loss, higher tumour grade, and right-sided location.[11] In our study, we did not find statistically significant correlation of CK20 staining and location of tumour process, T stage, nodal and distant metastases. However, we established a weak downhill relationship between CK20 expression and histologic grade (p=0.002, Kendall's tau-b = −0.323).

CONCLUSIONS

The present study showed that loss of CDX2 expression is associated with histologic grade and depth of tumour invasion (or T pathologic stage.) CK20 positivity is associated with histologic grade. No association between CK7 expression and histopathologic features was established. Colorectal carcinomas were divided in 4 classes depending on their positivity for CK20 and CK7. In 35.3% of the cases deviations from the standard CK20+/CK7− immunoprofile were observed. Aberrant cytokeratin immunostaining should always be considered when used in diagnosis of metastatic colorectal carcinoma.

Our results may help in demonstrating the heterogeneity of colorectal carcinomas. Further investigation on bigger cohorts is needed for better understanding the correlations between different immunohistochemical cytokeratin profiles and morphologic, clinicopathologic, molecular, and prognostic characteristics of colorectal carcinomas.

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Иммуногистохимическая экспрессия CK20, CK7 и CDX2 при колоректальной карциноме в корреляции с патоморфологическими характеристиками

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Резюме

Введение: Колоректальная карцинома является третьим наиболее распространённым видом рака в мире. Обычный иммунофенотип колоректальной аденокарциномы положительный по CDX2, положительный по CK20 и отрицательный по CK7. Сообщается об аберрантной экспрессии в различных колоректальных карциномах, но её связь с морфологическими переменными и данными о выживании до сих пор неясна.

Цель: Целью этого исследования было изучение корреляции между аберрантным иммуноокрашиванием колоректальной карциномы и различными клинико-патологическими характеристиками.

Материалы и методы: Иммуногистохимическая экспрессия CK20, CK7 и CDX2 оценивалась в 71 случае колоректальной карциномы. Для выявления корреляции между морфологическими характеристиками и иммунопрофилем колоректальной карциномы был проведён статистический анализ.

Результаты: Положительный цитоплазматический и/или мембранный сигнал для CK20 наблюдался в 66.2 % колоректальных карцином. Положительное иммуноокрашивание на CK7 наблюдалось в 7% случаев. Что касается комбинированной экспрессии CK20 и CK7, доля иммунопрофиля CK20+/CK7- была самой высокой, составляя 46 из 71 колоректальной карциномы, за которыми следовали CK20-/CK7-, затем CK20-/CK7+ и CK20+/CK7+. Что касается CDX2, то большинство колоректальных карцином (87.3%) показали положительное окрашивание. Была установлена статистически значимая корреляция между экспрессией CDX2 и гистологической степенью и глубиной инвазии опухоли. Потеря положительности CK20 была связана с более высокой степенью гистологической оценки. Не было установлено никакой связи между экспрессией CK7 и гистопатологическими особенностями.

Заключение: Результаты подтверждают гетерогенность колоректального рака. Более 35% случаев в этом исследовании показали отклонения от ожидаемого иммунного профиля. Это следует учитывать при диагностике колоректального рака в метастатических областях.

Ключевые слова

CRC, IHC, CK20, CK7, CDX2