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Original Article

Immunohistochemical Phenotype of Colorectal Carcinoma in Patients with KRAS Mutation and Mismatch Repair Status

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Abstract

Introduction: Aberrant expression of CK7/CK20/CDX2 is reported in percentage of colorectal carcinomas (CRC).

Aim: The objective of this study was to investigate specific morphological and immunohistochemical characteristics of colorectal carcinoma with KRAS mutation status and microsatellite instability.

Materials and methods: Seventy-one patients with CRC and examined KRAS mutation status were included in the investigation. Immunohistochemistry was performed using antibodies to CK7, CK20, CDX2, PMS2, and MSH6. An automatic immunostainer (Ventana BenchMark GX) was used following the manufacturer' instructions. Fisher's exact test was used for statistical analysis (p value <0.05).

Results: Immunohistochemical analysis was performed for CK7, CK20, CDX2, PMS2, and MSH6. Aberrant expression of the typical immunohistochemical profile CK7/CK20/CDX2 was observed in 50% of the cases. The highest sensitivity and specificity were established for CDX2, with 93% of the cases demonstrating positive nuclear expression in the tumor cells. As for the microsatellite status, 20% of the examined colorectal cancers showed loss in expression for one or both of the mismatch repair proteins - PMS2 and MSH6, which was associated with loss of expression for CK20 and CDX2 as well. Downhill correlation was found also between CK20 expression and the presence of mutation in the gene for KRAS.

Conclusions: Our results may support the heterogeneity of colorectal carcinoma. Statistically significant correlation was found between the expression of CK20 and CDX2 and microsatellite deficient and KRAS mutant colorectal cancers. This may lead to application of immunohistochemical screening panel for selection of patients with CRC for genetic testing. Further studies on large cohorts correlating different immunohistochemical profiles to molecular subtypes of colorectal carcinoma are needed for better understanding of the pathogenesis and behavior of colorectal carcinoma.

Keywords

colorectal carcinoma, CRC, MSI, KRAS

INTRODUCTION

Colorectal carcinoma (CRC) is the third most common cancer worldwide and the third leading cause of cancer-related death in both sexes. CRC is the result of the gradual accumulation of genetic and epigenetic alterations that lead to the transformation of the normal mucosa into neoplastic tissue. APC, KRAS, p53, and MSI are the most frequently affected genes. Mutations in KRAS have prognostic and predictive value for the response to the therapy with anti-EGFR inhibitors. Microsatellite instability accounts for 15%-20% of colorectal adenocarcinomas. These tumors show some morphological and immunohistochemical features, have better prognosis and are resistant to treatment with 5-fluorouracil. The most widely used immunohistochemical markers for colorectal carcinoma are CDX2, CK20, and CK7. The characteristic immunoprofile is considered to be CDX2+/CK20+/CK7– with aberrant expression reported in a percentage of colorectal carcinoma with high MSI.[1,2]

AIM

The aim of this study was to investigate specific morphological and immunohistochemical features of colorectal carcinoma with KRAS mutation status and microsatellite instability.

MATERIALS AND METHODS

Seventy-one patients who have undergone hemicolectomies due to colorectal cancer between January 2017 and December 2018 were retrospectively included in this study. All of them were examined for KRAS mutation status. Paraffin-embedded tissue sections were collected from archives and the materials were reviewed by two pathologists to confirm the diagnosis and evaluate the histological type, differentiation, and depth of invasion. WHO criteria were used for histological typing. Postoperative pathologic staging was performed according to American Joint Committee on Cancer (AJCC) TNM staging system.

Immunohistochemistry was performed for protein markers CDX2, CK20, and CK7. For the purpose, 3-μm thick sections were cut from blocks of paraffin-embedded tissue. The antibodies used for the experiment were anti-CK 20 (SP33, Ventana, Roche) Rabbit Monoclonal Primary Antibody, anti-CK 7 (SP52, Ventana, Roche) Rabbit Monoclonal Primary Antibody, and nuclear protein CDX2 (EPR2764Y, Ventana, Roche). An automatic immunostainer (Ventana BenchMark GX) was used following the manufacturer's protocols.

To interpret 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 tumor cell, seen at 20× magnification

2+: there is intermediate cytoplasmic/membranous or nuclear expression in tumor cells, seen at low 10× magnification

3+: there is strong cytoplasmic/membranous or nuclear expression in tumor cells, seen at low 4× magnification

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

For evaluation of the mismatch repair status, immunohistochemical expression for PMS2 (A16-4, Ventana, Roche) and MSH6 (SP93, Ventana, Roche) was used. MMR proteins were defined as negative by the complete absence of IHC staining in the nucleus of tumor cells while normal cells remained stained, ensuring the technical validity of the experiment. Cases in which loss of one or both of the MMR proteins was observed were considered MMR-deficient, whilst those in which both of the MMR proteins were preserved – MMR-proficient.

For statistical analysis we used SPSS v. 19. Categorical variables were presented as number of patients (%), and normally distributed continuous variables were presented as mean and standard deviation. Fisher's exact test and the coefficient of correlation of Kendall's Tau-b, as an alternative, were used to estimate the correlations between CDX2, CK20, and CK7 expression and microsatellite and KRAS mutation status. A p value <0.05 was considered statistically significant. The Fisher's exact test was used and the coefficient of correlation of Kendall's Tau-b as an alternative **(Fig. 1)**.

RESULTS

Clinicopathological parameters of the patients

Detailed data are presented 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. CK7 positive immunostaining was seen in 7% of the cases. The majority of cases (87.3%) showed positive nuclear staining for CDX2. In terms of the combined expression of CK20 and CK7, the proportion of immunoprofile CK20+/CK7– was the highest, accounting for 46 out of 71 colorectal carcinomas.

KRAS mutation status

KRAS mutation was detected in 40 (56.3%) patients. Of these, 22 were men and 18 were women. It is worth of note

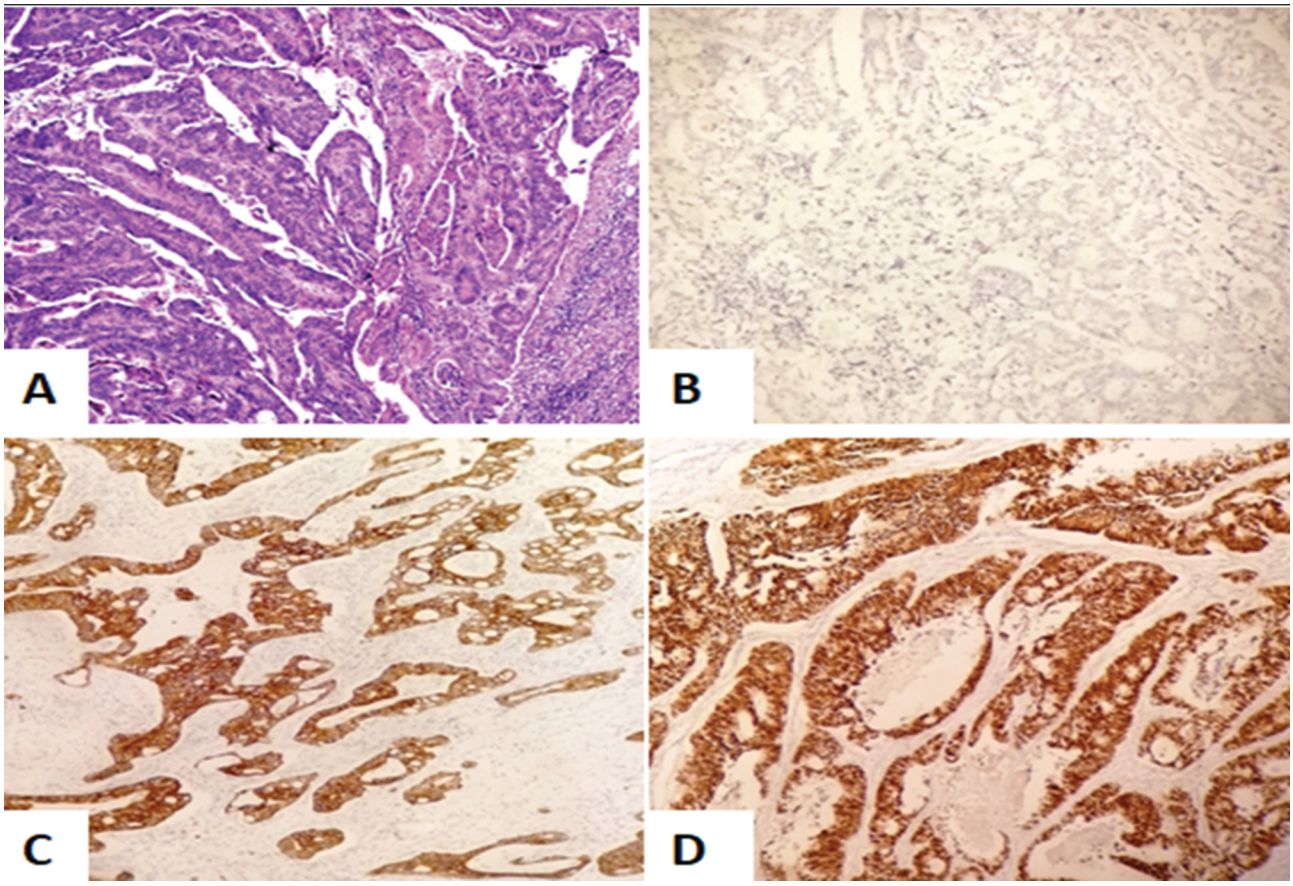

Figure 1. Histological and immunohistochemical characteristics. Conventional type colorectal carcinoma (**A**), Negative CK7 staining (**B**), Positive membranous signal for CK20 (**C**), Positive nuclear staining for CDX2 (**D**).

that in the under-50 age group, the mutation for KRAS was observed in 6 of 8 patients, whilst among the older patients the distribution of mutated vs. KRAS wild-type was approximately even. No correlations between KRAS mutation status and localization, histologic grade, and pathologic stage were observed.

Mismatch repair status

A mismatch repair (MMR) deficiency was found in 24 cases, six of which presented with losses in both proteins. From the remaining 18 loss form PMS2 alone was seen in 10 and loss for MSH6 alone – in 8 patients. MMR deficiency was evenly distributed among both sexes. In contrast to KRAS mutation status, all patients aged less than 50 years were MMR-proficient. No correlation between microsatellite status and localization, histologic grade, and pathologic stage was observed.

KRAS mutation status and CDX2, CK20, and CK7 expression

No statistically significant correlation was found between these.

Mismatch repair status and CDX2, CK20, and CK7 expression

Loss in CK20 expression was observed more frequently among MMR-deficient compared to MMR-proficient colorectal carcinomas (50% vs. 25%) (one-sided Fisher's exact test, p=0.037, Kendall's Tau-b correlation coefficient = −0.245). No statistically significant correlation was found between expression for CDX2, CK7 and the MMR status.

KRAS mutation and the mismatch repair status

The loss of protein expression for PMS2 was higher in KRAS wild-type colorectal carcinomas (Fisher's exact test, p=0.005, Kendall's Tau-b correlation coefficient = 0.341). No statistically significant correlation was observed between MSH6 protein expression and KRAS mutation status. MMR-proficient tumors were more likely to be KRAS-mutated (Fisher's exact test, p=0.02, Kendall's Tau-b correlation coefficient = −0.271)

Table 1. Clinicopathological parameters

Parameter	Number (%)
Sex	
Male	38 (53.5%)
Female	33 (46.5%)
Age (years)	
Mean	65 years
Minimum	37 years
Maximum	83 years
Standard deviation	11 years
Tumor 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 tumor	
T1	0
T2	3 (4.2%)
T3	39 (54.9%)
T4	23 (32.4%)
Missing data	6 (8.5%)

DISCUSSION

Colorectal carcinoma is a heterogeneous group of diseases with distinctive genetic and epigenetic alterations described by microsatellite instability (MSI) and chromosomal instability (CIN). KRAS is a member of the Ras family, first described as a cellular homolog of a transforming gene in the Kirsten rat sarcoma virus. It plays a key role in the intracellular signaling pathways and is a major prognostic factor.[3] MSI is determined by defects in the DNA mismatch repair system (MMR). MMR deficiency is caused by one or more mutations in MMR genes, such as MLH1, MSH2, PMS2, and MSH6. The MSI test and immunohistochemistry (IHC) are commonly used for screening for MMR deficiency, using four- or two-antibody panels. According to Kim et al. the use of a two-antibody panel PMS2 and MSH6 is sensitive and cost-effective screening for mismatch repair deficient colorectal tumors.[4]

In the present study, we performed immunohistochemical analyses for CK7, CK20, and CDX2 in 71 patients with colorectal carcinoma and examined their KRAS status. We stratified the cases into MMR-proficient and MMR-deficient using the two-antibody panel PMS2 and MSH6. We analyzed the immunohistochemical profile and the clinicomorphological characteristic according to the KRAS mutation and mismatch repair status.

The most widely used immunohistochemical markers for colorectal carcinoma are CK7, CK20, and CDX2. The characteristic immunoprofile is considered to be CDX2+/CK20+/CK7− with some aberrant expression reported in MSI.[1] In our study, 32 out of 71 cases showed aberrancies from the typical immunophenotype.

In the common literature, there are limited data concerning KRAS mutation status and CDX2, CK20, and CK7 expression. In our study, we found no statistically significant correlation between these parameters. Regarding the mismatch repair status, previous investigators have established links between MMR-deficient colorectal carcinomas and loss in expression for CDX2 and CK20.[5] Our results are in agreement with these statements for CK20. The proportion of CK20-negative CRCs in the group of MMR-deficient tumors was significantly higher than in the group of MMR-proficient tumors (50% vs. 25%, p=0.037). We found no relationship between CDX2 expression and the mismatch repair status, but think it is noteworthy that CDX2 expression was retained in all cases of MMR-deficient CRC with loss for both proteins, while the proportion of CDX2- negative cases was the highest in the group of MMR-deficient CRC with loss only for one mismatch repair protein (16.7%), followed by the group of MMR-proficient tumors (12.8%).

There are controversial data in the literature regarding the association between the KRAS mutation and the mismatch repair status. Ye et al. have stated that MMR-proficient tumors are more likely to be KRAS-mutated (n=535), whilst Grieken et al. have found higher KRAS-mutation rate amongst MMR-deficient CRCs (n=712). In our study, we received higher rates of KRAS-mutation amongst MMR-proficient tumors (77.5% in MSS vs. 22.5% in MSI, p=0.021). In 9 cases, both KRAS mutation and microsatellite instability were observed. Of these, only 1 presented with loss for both MMR proteins.[6,7]

CONCLUSIONS

In the era of personalized medicine, the pathological analysis that provides histologic and molecular information is critical to appropriate patient treatment, prognosis assessment, and family counseling. Further studies are needed for better understanding of all molecular mechanisms in tumorigenesis of CRC that will provide new target therapies, optimize the treatment, and benefit the prognosis of the patient.

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Иммуногистохимический фенотип колоректальной карциномы у пациентов с мутацией KRAS и статусом несоответствия репарации (Mismatch Repair Status)

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Резюме

Введение: Сообщается об аберрантной экспрессии CK7/CK20/CDX2 в процентах от колоректального рака (КРР).

Цель: Целью данного исследования было изучение специфических морфологических и иммуногистохимических характеристик колоректальной карциномы с мутационным статусом KRAS и микросателлитной нестабильностью.

Материалы и методы: В исследование был включен 71 пациент с КРР и исследованным мутационным статусом KRAS. Иммуногистохимию проводили с использованием антител к CK7, CK20, CDX2, PMS2 и MSH6. Автоматический иммуностейнер (Ventana BenchMark GX) использовали в соответствии с инструкциями производителя. Для статистического анализа использовали точный критерий Фишера (значение $p<0$).

Результаты: Был проведён иммуногистохимический анализ CK7, CK20, CDX2, PMS2 и MSH6. Аберрантная экспрессия типичного иммуногистохимического профиля CK7/CK20/CDX2 наблюдалась в 50% случаев. Наиболее высокая чувствительность и специфичность были установлены для CDX2, при этом в 93% случаев наблюдалась положительная ядерная экспрессия в опухолевых клетках. Что касается микросателлитного статуса, 20% исследованных колоректальных раков показали потерю экспрессии одного или обоих белков репарации несоответствия – PMS2 и MSH6, что также было связано с потерей экспрессии CK20 и CDX2. Нисходящая корреляция была обнаружена также между экспрессией CK20 и наличием мутации в гене KRAS.

Заключение: Наши результаты могут подтверждать гетерогенность колоректальной карциномы. Статистически значимая корреляция была обнаружена между экспрессией CK20 и CDX2 и колоректальным раком с дефицитом микросателлитов и мутантным KRAS. Это может привести к применению панели иммуногистохимического скрининга для отбора пациентов с КРР для генетического тестирования. Для лучшего понимания патогенеза и поведения колоректальной карциномы необходимы дальнейшие исследования на больших когортах, коррелирующие различные иммуногистохимические профили с молекулярными подтипами колоректальной карциномы.

Ключевые слова

колоректальный рак, CRC, MSI, KRAS