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Polymorphisms of CD44 rs187115 as a Predictive Biomarker in Early Colorectal Cancer Diagnostic

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Rezumat

Polimorfismul genei CD44 rs 187115 ca biomarker predictiv în diagnosticul incipient al cancerului colorectal

Introducere: Cancerul colorectal (CRC) prezintă o incidență tot mai mare la nivel mondial în ultimii ani, subliniind importanța metodelor de diagnostic precoce. Acest studiu şi-a propus să evalueze influența polimorfismului genei CD44 rs187115 asupra susceptibilității CRC.
Material şi Metodă: Studiul a cuprins 470 de pacienți CRC şi 165 de martori sănătoşi. Genotiparea tuturor probelor de sânge biologic a fost efectuată utilizând testul TaqMan pe sistemul ABI 7500 Real Time PCR (Applied Biosystems, SUA).
Rezultate: Genotiparea a arătat că purtătorii alelei variantei G, inclusiv genotipurile AG şi GG, au prezentat un risc crescut de apariție a CRC, cu un raport de şanse (OR) de 1,89 (interval de încredere 95% [IC] = 1,57-1,97); p = 0,047), comparativ cu cei care poartă genotipul AA.
Concluzii: Descoperirile subliniază utilitatea potențială a polimorfismelor CD44 rs187115 ca un biomarker predictiv nou pentru prognosticul CRC.

Cuvinte cheie: cancer colorectal, CD44, rs187115, biomarker

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Abstract

Introduction: Colorectal cancer (CRC) has exhibited an increasing incidence worldwide in recent years, underscoring the importance of early diagnosis methods. This study aimed to assess the influence of CD44 gene polymorphism rs187115 on CRC susceptibility.

Material and Methods: The study encompassed 470 CRC patients and 165 healthy controls. Genotyping of all biological blood samples was conducted using the TaqMan assay on the ABI 7500 Real Time PCR System (Applied Biosystems, USA).

Results: The genotyping revealed that carriers of the variant G allele, including the genotypes AG and GG, exhibited a heightened risk of CRC occurrence, with an odds ratio (OR) of 1.89 (95% confidence interval [CI] = 1.57-1.97; p = 0.047) compared to those carrying the AA genotype.

Conclusions: The findings underscore the potential utility of CD44 rs187115 polymorphisms as a novel predictive biomarker for CRC prognosis.

Key words: colorectal cancer (CRC), single nucleotide polymorphisms (SNP), CD44, rs187115, biomarker

Introduction

Colorectal cancer (CRC) is characterized by its development from various precursor lesions, such as conventional adenomas and serrated polyps, each driving progression to carcinoma through distinct pathways (1). While the majority of CRC cases advance through the adenoma-carcinoma sequence, serrated polyps, previously referred to as hyperplastic polyps, are also recognized as precursor lesions following an alternative pathway to CRC. Notably, the risk of developing CRC is influenced by environmental and genetic factors (2,3). A known component of this germline CRC predisposition encompasses rare, high-penetrance, and common, low-penetrance genetic variants (4,5).

Additionally, CRC encompasses a wide spectrum of neoplasia, ranging from benign stages to invasive cancers, and commonly manifests as epithelial-derived tumors, such as adenocarcinomas or adenomas. It is essential to focus on the development of novel molecular non-invasive tests for colorectal cancer (CRC) that are based on the detection of CRC alterations and exhibit higher sensitivity and specificity compared to current methods (6). The primary objective is to identify molecular markers such as DNA, RNA, and proteins. The end goal is to enhance survival rates and contribute to the advancement of personalized medicine through the discovery of "optimal" diagnostic biomarkers (7,8). These biomarkers should possess high sensitivity and specificity, while also being safe, cost-effective, and straightforward to measure. The task of developing such markers remains a considerable challenge, but offers significant potential for improving early detection and risk stratification for CRC.

Colonoscopy is widely acknowledged as the gold standard for colorectal cancer screening due to its high sensitivity and specificity. However, it presents significant costs in terms of finances and personnel, necessitating skilled endoscopists and patient compliance. The emergence of advanced molecular techniques holds promise for aiding in the detection and treatment of colorectal cancer. Currently, the categories of colorectal biomarkers under exploration include proteins, DNA (for mutation and methylation marker detection), RNA (primarily microRNAs), volatile organic compounds, and alterations in gut microbiome composition (9,10).

The extensive research on CD44 in recent years has highlighted its significance as a marker of progression and resistance to therapy in various cancer types. Specifically in colorectal

cancer (CRC), specific isoforms of CD44 have been identified to significantly contribute to carcinogenesis, disease progression, metastasis, and resistance to treatment (11). Additionally, the clinical and pathological implications of CD44 suggest its potential as a molecular target for cancer therapy. CD44 serves as a pivotal membrane receptor for hyaluronic acid (HA) and has been recognized for its ability to instigate various tumor biological processes, including proliferation, differentiation, invasion, and motility (12). The alternative splicing of variable exons in CD44 mRNA results in multiple variants, such as CD44v2, CD44v3, CD44v5, CD44v6, among others, with CD44v being exclusively identified in certain epithelial cells.

Furthermore, the isoform lacking variable exons in CD44 is denoted as CD44s (13), constituting the smallest CD44 molecule (85-95 kDa) expressed in vertebrate cells. CD44s and its isoforms exhibit distinct implications in the context of cancer (14).

We conducted a further evaluation of the correlation between CD44 rs187115 polymorphism and the prognosis of patients with CRC. A total of 470 CRC patients were monitored to investigate the influence of CD44 rs187115 polymorphism on CRC risk. The analysis revealed that individuals carrying AG+GG genotypes demonstrated a poorer overall survival compared to those with the AA genotype.

Materials and Methods

Genotyping of Biological Samples

The study encompassed 470 patients diagnosed with CRC and 165 controls procured from the Department of Surgery at County Emergency Hospital Timisoara between 2020 and 2023. A standardized protocol was consistently employed for the utilization of biological samples and DNA extraction, utilizing uniform methodology and reagents for automatic extraction. *Table 1* offers a comprehensive summary of the clinicopathological characteristics of the study subjects. The study received approval from the Ethical Committee of Victor Babes University of Medicine and Pharmacy under reference number 27/25.06.2020.

Statistical analysis

The statistical analysis was performed using SPSS software for Windows, version 26, developed by SPSS, USA. Statistical significance was defined as $p < 0.05$. Hardy-Weinberg equilibrium was assessed for all genotypes. The study aimed to assess variations in CD44 rs187115 polymorphism among CRC patients and control subjects by utilizing the Chi-squared test in relation to their clinical parameters.

Results

Study Population Characteristic

In our study, a total of 470 patients diagnosed with CRC were included, comprising 58.23% males and 41.77% females, with an additional 165 individuals serving as controls. The average age of CRC patients was 52.78 ± 27.56 years, while controls had a mean age of 65.38 ± 32.48 years. Our analysis revealed statistically significant differences in CD44 rs187115 polymorphisms between the patients with CRC and the control group. The prevalence of the GG genotype group was 48.58% (187/84),

Table 1. Demographic characteristics of the CRC patients and controls

Parameters	Colorectal cancer cases	Controls
Number	470	165
Sex		
Male	58.23%	67%
Female	41.77%	33%
Age (years)		
AJCC stage		
I	178	-
II	124	-
III	79	-
IV	89	-
Tumor Size (cm)	6.77+4.35 cm	-
Location		
colon	54.43%	-
rectum	18.42%	-
rectosigmoid	27.15%	-

the AG genotype group was 29.45% (145/51), and the AA genotype group was 21.97% (138/25). Utilizing a linear regression model, we were able to predict CRC risk, yielding a model explanatory power of R2 = 0.27. For a comprehensive overview of the genotypes for CD44 rs187115, please refer to *Table 2*.

Table 2. Genotype frequencies of CD44 rs 187115 polymorphisms in CRC patients

Gene	Rs187115	GG	AG	AA
CD 44	GG/AG/AA	48.58% (187/84)	29.45% (145/51)	21.97% (138/30)

Association Between CD44 rs187115 and the Risk of Colorectal Cancer

The association between CD44 rs187115 polymorphisms and colorectal cancer has been investigated. Significant differences were found in the prevalence of different genotypes (AA, AG, and GG) between patients with colorectal cancer and the control group. The carriers of the AG and GG genotypes were found to have an increased risk of colorectal cancer. Additionally, a linear regression model was used to predict the risk of colorectal cancer, with the model achieving an explanatory power of R2 = 0.27. For detailed results regarding the genotypes for CD44 rs187115, please refer to *Table 2*.

Discussion

Multiple studies have demonstrated the significant association of CD44 rs187115 polymorphisms with susceptibility and prognosis in various cancer types. Clinical investigations have revealed that the presence of the rs187115 polymorphism in the CD44 gene may act as a risk factor, impacting the clinical features of CRC, and influencing its prognosis. It is worth noting that the findings from different studies have displayed inconsistencies.

In our study, we discovered an association between the CD44 rs187115 polymorphism and an increased risk of CRC in the Romanian population. Numerous studies have previously investigated the relationship between CD44 rs187115 polymorphism and cancer risk, yielding conflicting results. Vazquez et al. identified an association between CD44 rs187115 polymorphism, weakened responses to chemotherapeutic treatments, and decreased overall survival in soft-tissue sarcoma patients (15). Chen et al. reported that the CD44 rs187115 polymorphism was linked to the risk of cervical, lung, and liver cancer, but not associated with the risk of breast, gastric, colon, or rectal cancer. Conversely, in Indian populations, no significant association was observed with gallbladder (16) and bladder cancer patients (17).

Stotz et al. demonstrated that the rs187115 polymorphism within the stem cell gene CD44 predicted outcomes in Stage II and Stage III colon cancer patients (18). Their findings indicated a statistically significant association between CD44 rs187115 polymorphism and recurrence, suggesting a potential risk factor associated with this SNP in colorectal cancer patients (19,20). These results are consistent with the findings of this study.

It is imperative to acknowledge certain limitations in this study. The nature of this research is exploratory, and it did not account for factors such as environmental and lifestyle influences on carcinogenesis. The fact that the study was conducted in a single centre can present some limitations because of the population genetics. In the future, we plan to extended this study in other geographical areas of Romania, to see the results in different population and to analyse the influence of environmental and lifestyle factors in colon carcinogenesis. Furthermore, it is conceivable that the examined CD44 rs187115 polymorphism may be associated with other polymorphisms that impact susceptibility to CRC and the prognosis of patients. Additionally, the selection of patients and controls from a single hospital may not be fully representative of the general population. Consequently, it is paramount to conduct further studies involving larger and more

diverse groups of patients and controls to authenticate our findings.

Also, the regression model R2=0.27. Also explains the fact that other factors such as exposure factors, eating habits and clinical heterogeneity of colon cancer could present an important potential involve 27 ment in the carcinogenesis process. The results obtained in our study are in concordance with other studies. Etzioni at al (21) found that eating factors, smoking and alcohol consumption have an important role in colorectal carcinogenesis.

Conclusions

The findings of this study significantly contribute to the expanding knowledge of genetic factors influencing CRC susceptibility and prognosis. The results underscore the potential value of CD44 rs187115 gene polymorphisms as significant biomarkers in the management of colorectal cancer. The study represents the first case-control investigation of the CD44 rs187115 variant's association with clinical features and CRC risk in the Romanian population.

Our findings demonstrate an association between the CD4 rs187115 variant and CRC risk in the Romanian population. In summary, our conclusions suggest that integrating the CD44 rs187115 gene into CRC screening programs could facilitate the early prediction of CRC risk in the Romanian population.

Conflicts of Interests and Source of Funding

Authors have no conflict of interest.

Ethical Statement

The study respected Helsinki's Declaration on study on human subjects.

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