BMC Cancer

RESEARCH Open Access

Suppressor protein plasma levels and inflammatory indices in colorectal cancer patients

Nino Chigvinadze[1,2], Ia Pantsulaia[2*], Teimuraz Lejava[1], Archil Aladashvili[2], Tsitsino Atamashvili[2], Nino Khvichia[1] and Khatuna Rekhviashvili[2]

Abstract

Colorectal cancer (CRC) is a multifactorial and age-related disease. Additionally, age, sex, and risk factors for developing CRC may include genetic, epigenetic, and immunologic characteristics and lifestyle habits. Simultaneous examination of gene mutations and their products is vital for determining patient prognosis and treatment. Therefore, we assessed APC, KRAS, and TP53 plasma levels; inflammatory indices; and KRAS mutations in CRC patients and evaluated their role in cancer progression. The study population consisted of colorectal cancer patients (40 patients: 16 women and 24 men). KRAS mutations were detected using real-time PCR; APC, KRAS, and TP53 protein levels were measured via ELISA. The results revealed that inflammatory indices (MLR, PLR, NLR) are increased in CRC patients, especially in those with advanced stages. TP53 protein levels were increased in patients with progressive cancer, whereas no significant difference was detected in the plasma levels of APC and KRAS. The G12V KRAS mutation was associated with a poor prognosis and high PLR values. Our findings reveal that inflammatory indices such as the MLR, PLR, and NLR are linked to TP53 and APC plasma levels and offer new insights into their role in the development and progression of CRC.

Keywords Colorectal cancer, Suppressor protein, Inflammatory indices, KRAS gene mutations

Introduction

Currently, colorectal cancer is the third most common oncological disease. According to Globocan (https://gco.iarc.fr/en), approximately 1,900,000 new cases of colorectal cancer (CRC) are detected annually worldwide, and deaths have exceeded 930,000. Notably, the mortality rate is the highest in Eastern Europe (www.who.int). Prognostic studies predict that the prevalence of colorectal cancer is increasing, and by 2040, the number of new cases will reach 3.2 million (63% increase), and the number of fatal cases will reach 1.6 million per year. In Georgia, from 2018 to 2021, the incidence of CRC was 55.44 per 100,000 people, and CRC was among the top five most common diseases in terms of mortality [1, 2].

Generally, CRCs are associated with age, and more than 90% of patients are 65–75 years old. Numerous studies have shown that the incidence of CRC depends on sex, and CRC is diagnosed in 60% of men and 40% of women. In addition to age and sex, risk factors for developing CRC may be genetic, epigenetic, immunologic or related to unhealthy lifestyle habits [1, 2–3]. CRC development is associated with changes in molecular mechanisms related to the expression of inflammatory markers,

*Correspondence:
Ia Pantsulaia
i.pantsulaia@tsmu.edu
[1]Department of Genetics, Ivane Javakhishvili Tbilisi State University, Tbilisi 0186, Georgia
[2]Vl. Bakhutashvili Institute of Medical Biotechnology, Tbilisi State Medical University, Tbilisi 0159, Georgia

suppressor genes, or oncogenes. Moreover, current studies have revealed that inflammatory indices (MLR, NLR, and PLR) may be related to the prognosis of colorectal cancer and are associated with the tumor stage. Molecular processes in the tumor microenvironment are very complicated, which is why a particular group of scientists suggest that damage to the genetic machinery in tumor cells leads to endless cell division and tumor development [4–8]. Many genes are known to be responsible for the development of CRC; furthermore, familial forms of CRC have been described. In particular, a high incidence of CRC development among patients with familial adenomatous polyposis (FAP) has been shown [9, 10]. Familial adenomatous polyposis (FAP) is an inherited disease caused by mutations in the APC gene that lead to polyps and, ultimately, the development of colon cancer. For patients who do not receive appropriate treatment at the initial stage, the risk of developing cancer increases and appears at an early age of up to 40 years. On the other hand, hereditary nonpolyposis colorectal cancer (HNPCC), otherwise known as "Lynch syndrome," is one of the most important risk factors for the development of colon cancer [11]. Under these conditions, the risk of developing cancer starts at the age of 50, and MLH1 and MSH2 are caused mainly by mutations in the MSH6, PMS2, or EPCAM genes. APC mutations are common in familial and sporadic cases of CRC, occurring in approximately 75–80% of cases [12]. The APC protein regulates the WNT signaling pathway, controls the stability of β-catenin, and affects WNT target genes such as c-myc [13, 14]. In addition to the APC gene, KRAS and p53 are involved in the formation of CRC. The mutation frequency of these genes is associated with the risk of developing the disease and is currently the subject of significant research [15, 16].

Notably, APC, KRAS, and TP53 mutations are crucial in colorectal cancer (CRC). In particular, 30–40% of colon cancer patients have a suppressor KRAS mutation. KRAS mutations are particularly associated with the formation of metastases, which are caused by uncontrolled cell proliferation accompanied by disruption of regular GTPase activity. The K-Ras gene encodes the p21ras protein, which is essential for transmitting signals from the cell membrane to various effector molecules inside the cell [17, 18–19].

TP53, a tumor suppressor gene, plays a crucial role in regulating cell growth and apoptosis. TP53 mutations are found in many CRC cases, especially in proximal and distal tumors [20, 21–22]. Interactions between APC and TP53 mutations are thought to be associated with genomic instability and chromosomal aneuploidy, which further contribute to cancer development [23, 24]. Thus far, many studies are ongoing that explore the microenvironment of CRC and the associated molecular mechanisms, particularly APC, KRAS, and TP53 gene mutations, for selecting treatment strategies and determining disease prognosis [23–27]. However, very little is known about the relationships between proteins (APC, KRAS, and TP53) and the development and progression of this disease. As survival rates for CRC patients depend on the tumor stage, early detection of cancer and appropriate treatment are crucial. Studying the microenvironment of CRC, gene mutations, and their products, as well as revealing their relationships with inflammatory markers, is vital [23–27]. Therefore, our study aimed to explore the connection between APC, KRAS, and TP53 plasma levels and inflammatory markers and evaluate their role in cancer progression and metastasis.

Materials and methods

The study population consisted of colorectal cancer patients (40 patients in total: 16 women and 24 men) and age matched healthy individuals (20 person). Data concerning clinical status, treatment options and plasma/tissue specimens were collected from 2022 to 2023 at the Clinic of the National Center for Surgery—New Life. All patients were new cases scheduled for surgical treatment after diagnosis. Patients underwent surgical resection, after which the tumor stage was assessed using tissue histological analysis (T4–19; T3–16; T2–6). At the time of tissue/blood collection, the patient had not received any treatment, including radiation or chemotherapy. The medical history of the patients was evaluated; a full panel of laboratory tests, including the MLR, PLR, and NLR indicators from the blood analysis; quantitative analysis of the APC, KRAS, and P53 proteins; and examination of KRAS point mutations by real-time PCR.

The study was conducted at the Vladimir Bakhutashvili Institute of Biotechnology of Tbilisi State Medical University and the Department of Genetics of Ivane Javakhishvili Tbilisi State University. Each individual's participation was voluntary. The informed consent form was taken from all the participants.

Tissue samples: This study included tissue samples from 40 patients. Immediately after collection, the tissue was fixed in formalin and embedded in paraffin blocks (FFPE). First, the tissue was stained for histological examination, and the tumor stage was assessed; then, DNA was extracted from the paraffin blocks to detect KRAS mutations.

DNA extraction

According to the manufacturer's protocol, genomic DNA was extracted from paraffin blocks (Quick-DNA Miniprep Plus Kit, Zymo Research, California). A total of 750 µl of xylene was added to the samples, which were incubated at room temperature for one hour. The samples were subsequently washed three times in different

ethanol concentrations (100%, 95%, and 75%) and finally with 1 ml of ddiH2O. The appropriate proteinase K mixture was prepared for the deparaffinized sample and incubated overnight at 55 °C (12–16 h) and then at 94 °C for – 20 min, followed by centrifugation, washing three times, and obtaining the desired DNA. The DNA concentration was measured via a Qubit® 3.0 fluorometer.

Real-time PCR was used to assess mutations in the KRAS gene via the Quant Studio real-time PCR system (codons – 12, 13, 59, 61, 117, and 146). PCR was performed according to the protocol provided by the manufacturer (EasyÒ KRAS, Italy).

ELISA

For the quantitative evaluation of the APC, KRAS, and P53 proteins in blood plasma, an immunoenzymatic method was used. The experiment was performed according to the protocol provided by the manufacturer (KRAS-AABIN6962518 GTPase KRAS (KRAS) ELISA Kit, P53-Tumor Protein P53 (TP53) ELISA Kit, APC-Adenomatous Polyposis Coli (APC) ELISA Kit, Mybiosource, Southern California, San Diego (USA). The results were measured at 450 nm using Synergy H1 Multi-Mode Microplate Reader (Biotek, USA). Lower Limit of Detection (LLD) was defined as the lowest protein concentration that could be differentiated from zero. Thus, the detection limit of The minimum detectable dose of human P53 was less than 2.34 pg/ml, for KRAS– 10pg/ml and for APC - less than 0.003 ng/mL. The range of detection for APC 0.01-10ng/mL, For TP53 9.38 pg/ml-600 pg/ml, for KRAS– 62.5 pg/ml– 2000pg/ml. The samples were analyzed in duplicate and the coefficient of variation (CV) did not exceed 5% (KRAS, TP53) and 10% (APC). The intra-assay variability for all studied cytokines were less than 8% (KRAS, TP53) and 12% (APC).

Statistical analysis

Statistical analyses were performed by Prism version 6 (GraphPad Software, Inc.). The analytical strategy included the following main steps: (1) descriptive statistics and (2) identification of statistically independent groups (factors) of dependent variables. The normal distributions of the studied parameters were assessed via the Kolmogorov–Smirnov and Lilliefors normality tests. A study of the properties of the characteristic distribution of the studied molecules revealed that some quantities deviated from the normal distribution. To ensure that specific rare observations were not excluded, we used ≥ 4 SDs as the range criterion. Initial values are presented as the means, standard deviations, minimums, and maximums. One-way ANOVA was used to determine the significance of differences between patients and healthy controls. A p value of 0.05 or less was considered statistically significant for all analyses.

Table 1 The baseline characteristics (mean ± std. dev.) of the studied traits according to sex

Parameters	Mean ± Std.Dev.			
	Total ($n=40$)	Men	Women	p
Age	64,68 ± 10,45	63,50 ± 9,54	65,57 ± 11,24	0.23
Weight	66,65 ± 6,45	62,44 ± 6,61	69,86 ± 4,13	0,01
BMI	22,78 ± 1,97	16,00 ± 2,56	21,00 ± 1,36	0,27
MLR	1,12 ± 1,38	1,37 ± 1.61	0,93 ± 1,18	0,34
PLR	30,07 ± 27,83	41,79 ± 37,50	21,15 ± 12,07	0,02
NLR	5,90 ± 6,28	7,09 ± 7,14	5,00 ± 5,54	0,32
KRAS	5634,81 ± 3048,44	5179,13 ± 2186,63	5398,54 ± 2516,03	0,73
APC	322,83 ± 367,13	349,48 ± 411,09	297,10 ± 324,36	0,59
Tp53	574,48 ± 936,95	295,70 ± 423,32	447,44 ± 1047,48	0,48

BMI, bone mass index; MLR, monocyte/lymphocyte ratio; NLR, neutrophil/lymphocyte ratio; PLR, platelet/lymphocyte ratio; NS, not significant; TP53, tumor suppressor protein; APC, adenomatous polyposis coli; KRAS, K-Ras protein

Table 2 Changes in inflammatory indices according to health status (0 - healthy) and tumor stage (2, 3, or 4)

Parameters	Mean ± Std.Dev.		
	Healthy ($n=20$)	Patients ($n=40$)	p
MLR	*0,174 + 0,058*	*1,120 + 1,378*	*0,003*
PLR	*7,868 + 2,026*	*30,074 + 27,827*	*0,001*
NLR	*1,745 + 0,562*	*5,903 + 6,277*	*0,004*

MLR– monocyte/lymphocyte ratio, NLR– neutrophil/lymphocyte ratio, PLR–platelet/lymphocyte ratio. All differences were statistically significant ($p < 0.01$)

Results

First, descriptive statistics of the studied parameters were performed, and each parameter was scanned according to sex and age. As shown in Table 1, no significant differences between the studied molecules and anthropometric parameters were detected by sex. However, there is a tendency for inflammatory indices to be slightly greater than those in women.

In the next step, we compared inflammatory indices (MLR, PLR and NLR) between healthy controls and colorectal cancer patients. All three parameters (MLR, PLR and NLR) are significantly higher in cancer patients than in healthy individuals, which indicates a strong inflammatory condition in patients and can be used as a marker of disease progression. A statistically significant difference ($p < 0.05$) in all indicators emphasized the reliability of using ratios in clinical evaluations (Table 2).

A statistically significant difference was also found in the protein level of p53 between patients (574,48 ± 936,95) and the healthy population (< 156,25 pg/ml, $p = 0.008$). Elevated levels of p53 protein indicate p53 gene activation and involvement in tumor development (Fig. 1). Moreover, the KRAS and APC protein levels did not differ from those in healthy individuals.

Additionally, to reveal the relationships between different clinical features and molecular parameters, several KRAS mutations—G12S, G12C, G13D, A59x, Q61x,

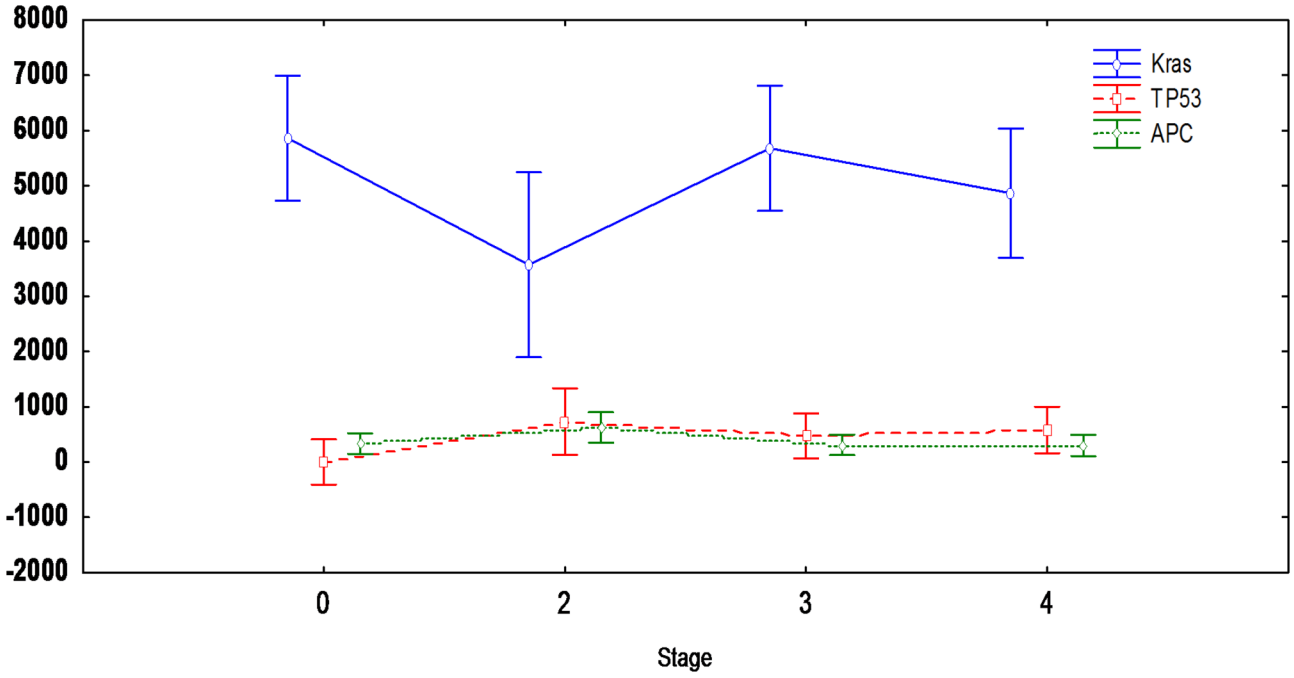

Fig. 1 Changes in the plasma levels of the supressor (KRAS, TP53) and APC proteins according to stage. TP53 - tumor suppressor protein, APC - adenomatous polyposis coli; KRAS - K-Ras protein

Table 3 The studied traits (mean ± standard deviation) were compared between K-ras mutation (G12V+) and nonmutation (G12V-) CRC patients

Parameter	Group 1 (G12V+) ($n=14$)	Group 2 (G12V-) ($n=26$)	*P* Value
Age (years)	68.000 ± 6.633	57.000 ± 14.758	0,063
Height (m)	1.704 ± 0.059	1.699 ± 0.081	0,459
Weight (kg)	66.857 ± 6.793	64.455 ± 8.092	0,693
BMI	23.042 ± 2.410	22.279 ± 1.784	0,382
MLR	1.193 ± 1.381	1.403 ± 1.753	0,579
PLR	17.816 ± 11.106	38.645 ± 37.283	0.008
NLR	7.228 ± 7.227	7.079 ± 7.824	0,968
KRAS	4980.273 ± 1744.167	4192.091 ± 1756.193	0,125
TP53	771.107 ± 826.763	550.519 ± 481.807	0,073
APC	458.771 ± 460.597	430.754 ± 441.818	0,899

BMI - bone mass index; MLR - monocyte/lymphocyte ratio; NLR - neutrophil/lymphocyte ratio; PLR - platelet/lymphocyte ratio; NS, not significant; TP53 - tumor suppressor protein; APC - adenomatous polyposis coli; KRAS, K-Ras protein

K117x, and A146x—were evaluated in the tissue material of the examined patients (20 samples). Among them, the most frequent G12V mutation was detected in 14 patients: 10 patients, stage T4; two patients, stage T3; and two patients, stage T2. Notably, two patients with the G12V mutation (30 and 26 years old, stage T4 and T2, respectively) died shortly after surgery.

The levels of inflammatory markers, as well as the concentrations of the APC, K-ras and P53 proteins, were subsequently evaluated in mutated (G12V) and nonmutated individuals (Table 3).

As shown in Table 3, the presence of the G12V mutation led to a higher PLR than did the absence of the mutation (38.645 vs. 17.816; $p<0.01$). However, no statistically significant differences in other parameters, including age, height, weight, BMI, MLR, NLR, TP53, and APC, were detected between the two groups. Additionally, there were no significant changes in the KRAS protein level in patients with KRAS gene mutations.

Finally, correlation analysis was performed between the plasma levels of inflammatory indices (MLR, PLR, and NLR) and cancer suppressor/activator molecules (KRAS, TP53, and APC). According to the results of the correlation analysis, inflammatory indices are interconnected. For example, the MLR is associated with the NLR ($r=0.832$, $p<0.01$) and the PLR ($r=0.492$, $p<0.05$). Notably, the PLR was also strongly negatively correlated with the APC (r = -0.601, $p<0.01$), indicating that the PLR is inversely related to APC expression and/or activity. A moderate negative correlation was found between the PLR and TP53 (r = -0.352, $p<0.05$), indicating a relationship between inflammatory indices and tumor suppressor genes. Moreover, KRAS, which represents mutations of the KRAS gene, shows only weak or moderate associations with other variables. The moderate negative correlation of KRAS with TP53 (r = -0.468, $p<0.05$) suggests a possible inverse interaction between KRAS mutations and TP53 activity, which may be crucial in cancer development.

Discussion

Colorectal cancer is a complex multifactorial disease, and many different factors are involved in its development. Studying the molecular mechanism of both the cancer microenvironment and the inflammatory indices associated with tumor onset is important [28, 29–30]. Therefore, our research focused on clarifying these issues.

First, we revealed statistically greater inflammatory ratios (MLR, NLR, and PLR) in patients than in healthy individuals. Although some variations were observed by sex—males had higher values than females did, statistically significant differences were not observed. In addition, when the inflammatory marker PLR was studied, high levels were associated with the stage of the tumor. Our results are in complete agreement with previous works that reported that the serum platelet count is considered a prognostic factor in CRC, in particular, with the findings of Gawinski et al., according to which colorectal cancer patients with low NLRs and low PLRs had a good prognosis and a better five-year survival (OS) rate [31, 32]. Thus, the determination of inflammatory markers is important for assessing the stage and severity of the disease and can be a step forward in personalized medicine.

To reveal the link between the cancer microenvironment and the immune system, simultaneous evaluation of inflammatory indices and cancer-associated genes or their synthesized products is necessary. Thus, we first investigated the plasma levels of tumor suppressor proteins (KRAS, P53) and APC in CRC patients [24, 33]. Previous studies have shown that tumor suppressor protein levels are associated with gene mutations, which are mainly responsible for CRC metastatic progression or treatment unresponsiveness [15, 34]. On the basis of our findings, we revealed that the plasma level of TP53 is elevated in colorectal patients compared with healthy individuals. Moreover, our data suggest that there is a connection between p53 protein levels and disease progression, as TP53 levels are increased, especially in stage III-IV patients who die shortly after surgery [21, 35]. Additionally, p53 levels are much higher in stage II patients, who relapse within a short period after surgery.

The p53 protein is rapidly degraded, with a half-life of 6–20 min. Under physiological conditions, p53 is degraded by ubiquitin-mediated proteolysis. The E3 ubiquitin-protein ligase Mdm2 (MDM2) protein is one of the central enzymes that labels p53 with ubiquitin, maintaining low expression of p53 under physiological conditions. Under cellular stress, *TP53* is activated, and p53 is overexpressed to induce cell cycle arrest, apoptosis and senescence. In addition to having direct implications for cells, p53 affects the surrounding microenvironment, controlling angiogenesis, cell migration and invasion.

These findings highlight p53 as a critical marker for evaluating tumor stage and prognosis in patients with colorectal cancer [36, 37–38]. Based our result, we speculate that TP53 plays a key role in colorectal cancer (CRC) progression by modulating inflammation and immune responses. Its loss or mutation leads to increased pro-inflammatory cytokines (IL-6, TNF-α, IL-1β), fostering chronic inflammation and tumor progression. TP53 inactivation also shifts macrophages toward an immune-suppressive M2 phenotype, increases the neutrophil/lymphocyte ratio (NLR), and enhances immune checkpoint molecule expression (e.g., PD-L1), contributing to immune evasion. Additionally, TP53 suppresses NF-κB, a major regulator of inflammatory pathways; its loss leads to persistent inflammation and resistance to apoptosis. Furthermore, mutant TP53 promotes tumor-derived secretions, including cytokine-rich exosomes and angiogenic factors (VEGF, HGF), supporting tumor growth and immune escape. Targeting TP53-mediated inflammatory pathways, such as NF-κB or IL-6 inhibitors, could offer new therapeutic strategies for CRC.

In contrast to TP53, no significant differences were detected in the circulating levels of the KRAS and APC proteins, which led us to study point mutations in the KRAS gene more thoroughly. Consequently, in the next step, several mutations (G12A, G12D, G12V, G12R, G12S, G12C, G13D, A59x, Q61x, K117x, and A146x) were screened. Notably, the G12V mutation was detected in 14 out of 20 patients, mostly at the T4 stage. Therefore, patients have a poor prognosis and fatal outcomes. Our results indicate that the value of the PLR is more than two times greater in patients with a mutation.

While the other markers (MLR and NLR) did not show significant differences between the groups with mutations, the elevated levels of the tumor suppressor protein TP53 protein in individuals with the G12V mutation may be have critical importance. The KRAS G12V mutation, known to affect immune checkpoint proteins such as PD-L1, plays a key role in immune surveillance and tumor development [39]. Previous studies have shown that KRAS-mutated cell lines exhibit increased PD-L1 activity and a positive correlation with immune response mechanisms [39]. Additionally, mutations in TP53 and KRAS activate specific markers associated with both antitumor immunity and immune tolerance [19, 38]. The aggressive nature of tumors with the KRAS G12V mutation is further supported by findings that these cells tend to metastasize more readily compared to cells with other KRAS mutations [40]. In cancers with TP53 mutations, particularly in combination with other oncogenic mutations such as KRAS, the tumor microenvironment becomes more immunosuppressive, with increased PD-L1 expression on cancer cells. This helps the tumor evade immune detection and promotes tumor progression. Therefore, taken together, these interactions suggest a complex triangle relationship between PD-L1, TP53,

and KRAS, which collectively contribute to shaping the oncogenic and immunological landscape of colorectal tumors. This interaction may help explain the particularly aggressive phenotype observed in tumors with the KRAS G12V mutation, which are more likely to metastasize and exhibit a more immunosuppressive microenvironment than tumors with other KRAS variants [40].

While the present findings provide valuable insights into the roles of TP53 and KRAS mutations in the development and progression of colorectal cancer (CRC), and contribute meaningfully to the existing knowledge, several limitations must be acknowledged. First, the relatively small sample size—particularly within the KRAS mutation subgroup—restricts the statistical power of the analysis and limits the generalizability of the results. Second, the absence of data on additional clinically and molecularly relevant variables, such as microsatellite instability (MSI) status, BRAF mutation status, and tumor anatomical location, hinders a more comprehensive stratification of the study population. Third, the cross-sectional design of the study precludes the establishment of causal relationships between inflammatory biomarkers and the expression of TP53 and APC. Finally, the lack of longitudinal follow-up data prevents an evaluation of the prognostic significance of TP53 expression and inflammation-related indices over time.

To address these limitations, future research should incorporate multi-omics approaches, larger and more diverse patient cohorts, and prospective longitudinal designs. In particular, functional studies investigating the effects of TP53 and APC mutations on the modulation of the tumor immune microenvironment may yield crucial insights into the mechanisms underlying immune evasion in CRC.

Conclusions

In conclusion, the PLR and TP53 levels were significantly greater in CRC patients than in healthy individuals. Additionally, the correlations among the inflammatory indices (MLR, NLR, and PLR) and between the PLR and the APC were statistically significant. Our findings reveal that inflammatory indices such as the MLR, PLR, and NLR are linked to TP53 and APC plasma levels and offer new insights into their role in the development and progression of CRC.

Abbreviations

APC	adenomatous polyposis coli
BMI	bone mass index
CRC	Colorectal cancer
KRAS	K-Ras protein
MLR	monocyte/lymphocyte ratio
NLR	neutrophil/lymphocyte ratio
PLR	platelet/lymphocyte ratio
NS	not significant
TP53	tumor suppressor protein

Acknowledgements

We thank all the participants and collaborators.

Author contributions

Author Contributions: N. Ch. collect the performed protein and KRAS gene mutation analysis and conceived of the presented idea. I. P. developed the main research plan, supervised immunological direction of study, performed the analytic calculations and write MS. Tinatin Chikovani contributed to the interpretation of the results. A. A., N. K. and K. R. supervised the collection of clinical data. Ts. A. measure all proteins by ELISA. All authors discussed the results and contributed to the final manuscript.

Funding

This work was supported by the Shota Rustaveli National Science Foundation (SRNSF) [PHDF-21-1886].

Data availability

The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

The study was approved by Biomedical Research Ethics Committee of Tbilisi State Medical University (meeting N4-2023/105). Informed consent to participate was obtained from all individual participants included in the study. Each individual's participation was voluntary. The research was performed in accordance with the Declaration of Helsinki and all methods were carried out following relevant guidelines and regulations.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Received: 15 October 2024 / Accepted: 22 April 2025
Published online: 25 April 2025

References

1. Nonikashvili M, Kereselidze M, Toidze O, Beruchashvili T. The incidence of colorectal cancer (CRC) in Georgia continues to increase: A population-based registry study. Cancer Treat Res Commun. 2023. https://doi.org/10.1016/j.ctarc.2023.100693.
2. Bray F, Laversanne M, Sung H, et al. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2024. https://doi.org/10.3322/caac.21834.
3. Sawicki T, Ruszkowska M, Danielewicz A, Niedźwiedzka E, Arłukowicz T, Przybyłowicz KE. A review of colorectal Cancer in terms of epidemiology, risk factors, development, symptoms and diagnosis. Cancers (Basel). 2021. https://doi.org/10.3390/cancers13092025.
4. Stojkovic Lalosevic M, Pavlovic Markovic A, Stankovic S, et al. Combined diagnostic efficacy of Neutrophil-to-Lymphocyte ratio (NLR), platelet-to-Lymphocyte ratio (PLR), and mean platelet volume (MPV) as biomarkers of systemic inflammation in the diagnosis of colorectal Cancer. Dis Markers. 2019. https://doi.org/10.1155/2019/6036979.
5. Zhang J, Zhang HY, Li J, Shao XY, Zhang CX. The elevated NLR, PLR and PLT May predict the prognosis of patients with colorectal cancer: a systematic review and meta-analysis. Oncotarget. 2017. https://doi.org/10.18632/oncotarget.18575.
6. Heshmat-Ghahdarijani K, Sarmadi V, Heidari A, Falahati Marvasti A, Neshat S, Raeisi S. The neutrophil-to-lymphocyte ratio as a new prognostic factor in cancers: a narrative review. Front Oncol. 2023. https://doi.org/10.3389/fonc.2023.1228076.
7. Kim J, Lee HK. Potential role of the gut Microbiome in colorectal Cancer progression. Front Immunol. 2022. https://doi.org/10.3389/fimmu.2021.807648.

8. Plundrich D, Chikhladze S, Fichtner-Feigl S, Feuerstein R, Briquez PS. Molecular mechanisms of tumor Immunomodulation in the microenvironment of colorectal Cancer. Int J Mol Sci. 2022. https://doi.org/10.3390/ijms23052782.
9. Jasperson KW, Tuohy TM, Neklason DW, Burt RW. Hereditary and Familial colon cancer. Gastroenterology. 2010. https://doi.org/10.1053/j.gastro.2010.01.05.
10. Al-Sukhni W, Aronson M, Gallinger S. Hereditary colorectal cancer syndromes: Familial adenomatous polyposis and Lynch syndrome. Surg Clin North Am. 2008. https://doi.org/10.1016/j.suc.2008.04.012.
11. Andersen SD, Liberti SE, Lützen A, et al. Functional characterization of MLH1 missense variants identified in Lynch syndrome patients. Hum Mutat. 2012. https://doi.org/10.1002/humu.22153.
12. Erazo-Oliveras A, Muñoz-Vega M, Mlih M, et al. Mutant APC reshapes Wnt signaling plasma membrane nanodomains by altering cholesterol levels via oncogenic β-catenin. Nat Commun. 2023. https://doi.org/10.1038/s41467-023-39640-w.
13. Lewandowska A, Rudzki G, Lewandowski T, Stryjkowska-Góra A, Rudzki S. Risk factors for the diagnosis of colorectal Cancer. Cancer Control. 2022. https://doi.org/10.1177/10732748211056692.
14. Tariq K, Ghias K. Colorectal cancer carcinogenesis: a review of mechanisms. Cancer Biol Med. 2016. https://doi.org/10.28092/j.issn.2095-3941.2015.0103.
15. Malki A, ElRuz RA, Gupta I, Allouch A, Vranic S, Al Moustafa AE. Molecular mechanisms of Colon cancer progression and metastasis: recent insights and advancements. Int J Mol Sci. 2020. https://doi.org/10.3390/ijms22010130.
16. Kudryavtseva AV, Lipatova AV, Zaretsky AR, et al. Important molecular genetic markers of colorectal cancer. Oncotarget. 2016. https://doi.org/10.18632/oncotarget.9796.
17. László L, Kurilla A, Takács T, et al. Recent updates on the significance of KRAS mutations in colorectal Cancer biology. Cells. 2021. https://doi.org/10.3390/cells10030667.
18. Porru M, Pompili L, Caruso C, et al. Targeting KRAS in metastatic colorectal cancer: current strategies and emerging opportunities. J Exp Clin Cancer Res. 2018;37:57.
19. Pantsar T. The current Understanding of KRAS protein structure and dynamics. Comput Struct Biotechnol J. 2019. https://doi.org/10.1016/j.csbj.2019.12.004.
20. Olivier M, Hollstein M, Hainaut P. TP53 mutations in human cancers: origins, consequences, and clinical use. Cold Spring Harb Perspect Biol. 2010. https://doi.org/10.1101/cshperspect.a001008.
21. Michel M, Kaps L, Maderer A, Galle PR, Moehler M. The role of p53 dysfunction in colorectal Cancer and its implication for therapy. Cancers (Basel). 2021. https://doi.org/10.3390/cancers13102296.
22. Liu Y, Zhang X, Han C et al. TP53 loss creates therapeutic vulnerability in colorectal cancer [published correction appears in nature. 2021, https://doi.org/10.1038/s41586-021-03664-3]
23. Thota R, Yang M, Pflieger L, et al. APC and TP53 mutations predict cetuximab sensitivity across consensus molecular subtypes. Cancers (Basel). 2021. https://doi.org/10.3390/cancers13215394.
24. Conlin A, Smith G, Carey FA, Wolf CR, Steele RJ. The prognostic significance of K-ras, p53, and APC mutations in colorectal carcinoma. Gut. 2005. https://doi.org/10.1136/gut.2005.066514.
25. Berney CR, Fisher RJ, Yang J, Russell PJ, Crowe PJ. Protein markers in colorectal cancer: predictors of liver metastasis. Ann Surg. 1999. https://doi.org/10.1097/00000658-199908000-00007.
26. Kuppusamy P, Govindan N, Yusoff MM, Ichwan SJA. Proteins are potent biomarkers to detect colon cancer progression. Saudi J Biol Sci. 2017. https://doi.org/10.1016/j.sjbs.2014.09.017.
27. Sun J, Zhao J, Jiang F, et al. Identification of novel protein biomarkers and drug targets for colorectal cancer by integrating human plasma proteome with genome. Genome Med. 2023. https://doi.org/10.1186/s13073-023-01229-9.
28. Christodoulides N, Lami M, Malietzis G, Rasheed S, Tekkis P, Kontovounisios C. Sporadic colorectal cancer in adolescents and young adults: a scoping review of a growing healthcare concern. Int J Colorectal Dis. 2020. https://doi.org/10.1007/s00384-020-03660-5.
29. La Rocca A, De Gregorio V, Lagreca E, Vecchione R, Netti PA, Imparato G. Colorectal Cancer bioengineered microtissues as a model to replicate Tumor-ECM crosstalk and assess drug delivery systems in vitro. Int J Mol Sci. 2023;24(6):5678. https://doi.org/10.3390/ijms2406567.
30. Novoa Díaz MB, Martín MJ, Gentili C. Tumor microenvironment involvement in colorectal cancer progression via Wnt/β-catenin pathway: providing Understanding of the complex mechanisms of chemoresistance. World J Gastroenterol. 2022. https://doi.org/10.3748/wjg.v28.i26.3027.
31. Gawiński C, Mróz A, Roszkowska-Purska K, et al. A prospective study on the roles of the Lymphocyte-to-Monocyte ratio (LMR), Neutrophil-to-Lymphocyte ratio (NLR), and Platelet-to-Lymphocyte ratio (PLR) in patients with locally advanced rectal Cancer. Biomedicines. 2023. https://doi.org/10.3390/biomedicines11113048.
32. Kang Y, Zhu X, Lin Z, et al. Compare the diagnostic and prognostic value of MLR, NLR and PLR in CRC patients. Clin Lab. 2021. https://doi.org/10.7754/Clin.Lab.2021.201130.
33. Testa U, Pelosi E, Castelli G. Colorectal cancer: genetic abnormalities, tumor progression, tumor heterogeneity, clonal evolution and tumor-initiating cells. Med Sci (Basel). 2018. https://doi.org/10.3390/medsci6020031.
34. Magnusson MI, Agnarsson BA, Jonasson JG, et al. Histopathology and levels of proteins in plasma associate with survival after colorectal cancer diagnosis. Br J Cancer. 2023. https://doi.org/10.1038/s41416-023-02374-z.
35. Lin L, Mi Y, Li X, et al. Higher plasma concentration of TP53-induced Glycolysis and apoptosis regulator is associated with a lower risk of colorectal cancer metastasis. Cancer Manag Res. 2018. https://doi.org/10.2147/CMAR.S190272.
36. Yu DH, Li JH, Wang YC, Xu JG, Pan PT, Wang L. Serum anti-p53 antibody detection in carcinomas and the predictive values of serum p53 antibodies, carcino-embryonic antigen and carbohydrate antigen 12– 5 in the neoadjuvant chemotherapy treatment for III stage non-small cell lung cancer patients. Clin Chim Acta. 2011. https://doi.org/10.1016/j.cca.2011.01.028.
37. Albitar M, Sudarsanam S, Ma W, et al. Correlation of MET gene amplification and TP53 mutation with PD-L1 expression in non-small cell lung cancer. Oncotarget. 2018. https://doi.org/10.18632/oncotarget.24455.
38. Dong ZY, Zhong WZ, Zhang XC, et al. Potential predictive value of TP53 and KRAS mutation status for response to PD-1 Blockade immunotherapy in lung adenocarcinoma. Clin Cancer Res. 2017. https://doi.org/10.1158/1078-0432.CCR-16-2554.
39. Pan LN, Ma YF, Li Z, Hu JA, Xu ZH. KRAS G12V mutation upregulates PD-L1 expression via TGF-β/EMT signaling pathway in human non-small-cell lung cancer. Cell Biol Int. 2021. https://doi.org/10.1002/cbin.11524.
40. Alamo P, Gallardo A, Di Nicolantonio F, et al. Higher metastatic efficiency of KRAS G12V than KRAS G13D in a colorectal cancer model. FASEB J. 2015. https://doi.org/10.1096/fj.14-262303.

Publisher's note