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Original article

BRAF MUTATION IN COLORECTAL CARCINOMA IS ASSOCIATED WITH TUMOR DEPTH, LOCATION, GRADE AND PD-L1 EXPRESSION: SINGLE CENTER EXPERIENCE

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Abstract

Introduction: BRAF mutations in colorectal carcinoma (CRC) are a known marker of poor prognosis and aggressive tumor behavior.

Aim: This study aimed to evaluate the correlation of BRAF mutations with tumor depth, anatomical location, histological grade, and programmed death-ligand 1 (PD-L1) expression in colorectal carcinoma.

Material and methods: A retrospective prospective analysis was conducted on 152 cases of CRC diagnosed at the Clinical Hospital Acibadem - Sistina. Tumor samples were tested for BRAF mutations and immunohistochemically stained for PD-L1 expression (clone SP263). Tumor depth and location were documented, and histological grades were determined. PD-L1 expression was assessed at cut-offs of 1-10%, 10-50%, and 50-100% of positive tumor cells.

Results: BRAF mutations were identified in 7.24% of cases, predominantly in right-sided colon tumors. Mutated cases exhibited greater tumor depth and higher histological grade (G3) compared to BRAF wild-type tumors. PD-L1 expression (50-100%) was significantly associated with BRAF-mutated tumors, particularly in advanced stages (IIIC and IVA). These tumors showed a higher likelihood of being located in the right colon and were linked to poorer differentiation and increased immune checkpoint expression.

Conclusion: BRAF mutations in CRC are associated with aggressive tumor characteristics, including greater depth, high grade, and right-sided location. The strong correlation with PD-L1 expression suggests potential therapeutic benefits of immune checkpoint inhibitors in BRAF-mutated CRC cases. Early identification of these mutations is crucial for optimizing patient outcomes.

Keywords: colorectal cancer, PD-L1, BRAF mutation

Introduction

BRAF gene mutations have long been recognized as significant drivers in various cancers, including colorectal carcinoma (CRC). In recent years, these alterations have gained importance as they have strong implications in the personal care of patients with metastatic

colorectal carcinoma (mCRC)[1]. BRAF mutations occur in approximately 5-10% of all CRC cases. The most common mutation is the V600E substitution, which leads to constitutive activation of the mitogen-activated protein kinases (MAPK) pathway, promoting cell proliferation and metastasis. BRAF-mutated CRCs often display distinct clinicopathological features, including proximal location, poor differentiation, mucinous histology, and microsatellite instability (MSI-H)[2,3].

PD-L1 Expression in CRC

Programmed death-ligand 1 (PD-L1) is a key immune checkpoint molecule that binds to programmed cell death protein 1 (PD-1), leading to immune evasion by tumor cells. Recent studies have shown that PD-L1 expression may serve as a significant prognostic marker in rectal carcinoma. High levels of PD-L1 have been associated with poorer overall survival and disease-free survival in patients with rectal carcinoma. This has sparked interest in the potential use of PD-L1 expression as a predictive biomarker for immunotherapy in this patient population.

Furthermore, research has indicated that assessing PD-L1 expression in rectal carcinoma may help identify patients who are more likely to benefit from immune checkpoint inhibitor therapy. As a result, integrating PD-L1 expression analysis into clinical management of rectal carcinoma holds promise for guiding treatment decisions and improving patient outcomes.

PD-L1 expression in CRC is heterogeneous, occurring in approximately 20-60% of cases. Its presence correlates with adverse clinicopathological characteristics, including advanced stage, lymph node metastasis, and poor prognosis[4].

Association between BRAF mutation and PD-L1 expression

There are studies that have unveiled a potential link between BRAF mutation status and PD-L1 expression in CRC. Specifically, BRAF-mutated CRCs exhibit higher levels of PD-L1 expression compared to BRAF wild-type counterparts. This association suggests a potential molecular interplay between BRAF-driven oncogenesis and immune evasion mechanisms mediated by PD-L1[5].

Clinical implications

The association between BRAF mutation and PD-L1 expression holds significant clinical implications. Firstly, it suggests that BRAF-mutated CRCs may be particularly susceptible to immunotherapy targeting the PD-1/PD-L1 axis. Clinical trials evaluating immune checkpoint inhibitors (ICIs) in this subset of patients have shown promising results, with improved response rates and survival outcomes compared to traditional chemotherapy. Additionally, the detection of PD-L1 expression in BRAF-mutated CRCs may serve as a predictive biomarker for selecting patients who are most likely to benefit from immunotherapy.

The aim of this study was to evaluate the correlation of BRAF mutations with tumor depth, anatomical location, histological grade, and programmed death-ligand 1 (PD-L1) expression in colorectal carcinoma.

Materials and methods

This retrospective-prospective study was conducted at the Department of Histopathology and Cytology at the Clinical Hospital Acibadem - Sistina. Tissue samples from surgical specimens from 152 patients diagnosed with CRC were analyzed. Informed consent was obtained from all patients or their relatives.

Working protocol

Surgical specimens were formalin-fixed at room temperature, macroscopically analyzed and routinely sampled. A standard number of samples were taken from resection margins, tumor tissue and regional lymph nodes. The macroscopic description of surgical material included dimensions of the resection, size of the tumor and its localization in relation to resection margins, and number of isolated regional lymph nodes.

Analysis by light microscopy

Microscopic analysis was performed on a light microscope with standard tissue cuts of 4-5μ thickness applied to the subject glass and stained with hematoxylin and eosin (HE). The microscopic description included: histological type of the tumor, degree of histological differentiation, nuclear grade, depth of tumor invasion, lympho-vascular invasion and number of positive lymph nodes. The stage of the disease was determined according to UICC, 8th ed (Union for International Cancer Control) criteria[6].

Molecular analysis

BRAF mutations were analyzed on paraffin tissue cuts processed with Cobas z 480, real time PCR for automated amplification using BRAF/NRAS mutation test (LSR).

Immunohistochemical analysis

Immunohistochemical analysis for PDL-1 was performed only on metastatic cases (n=90), on a tissue microarray, using clone SPF263, Ventana USA. PD-L1 expression was assessed at cut-offs of 1-10%, 10-50%, and 50-100% of positive tumor cells.

Statistical analysis

The statistical analysis of data was carried out using the statistical program SPSS 23.0. The Kolmogorov-Smirnov test and Shapiro Wilk's test were used to test the normality of data distribution. Categorical variables were displayed with absolute and relative numbers, and quantitative variables with mean, standard deviation, minimum and maximum values, median value and interquartile rank, depending on the tested distribution. For comparing categorical variables, the Chi-square and Fisher's exact test were used, while quantitative variables were compared with the Student's t-test. Statistical significance was defined at a level of $p<0.05$.

Results

BRAF correlation with demographic and pathohistological characteristics

BRAF mutations were found in 11 patients (7.24%). The mean age of patients with BRAF mutations was 72.1 ± 12.7 years, patients without mutations had a mean age of 68.5 ± 11.3 years; the difference in mean age of patients with/without BRAF mutations was statistically insignificant (p=0.32), although all 10 patients with BRAF mutation were older than 50 years. Fifty-four percent of BRAF positive patients and 65% of BRAF negative patients were males (Table 1).

BRAF-positive and BRAF-negative patients differed significantly concerning the T category of the tumor, i.e. tumor depth (p=0.009), shown in Table 2. Comparison of the two groups for individual T categories showed a significantly lower prevalence of T3 category tumors in the BRAF positive group (27.27% *vs* 65.96%, p=0.01), and a significantly higher prevalence of 4b category tumors (45.45% *vs* 8.51%, p=0.0002). BRAF mutation status was significantly different depending on the differentiation of the tumor (p=0.022). Patients with mutations were significantly less likely to have G2 category tumors, or moderately

Table 1. BRAF mutations in correlation with demographic characteristics

Variable	n	BRAF positive n (%)	negative n (%)	p-level
gender				
males	98	6(54.55)	92(65.25)	$X^2 = 0.5$
females	54	5(45.45)	49(34.75)	p = 0.475
age				
n				t = 0.99
mean ± SD		72.1 ± 12.7	68.5 ± 11.3	p = 0.32
min- max		43 - 87	34 - 86	
age groups				
≤50	15	1(9.09)	14(9.93)	Fisher's exact test
>50	137	10(90.91)	127(90.07)	p = 1.0

X^2 (Chi-square test), t(Student t-test)

Table 2. BRAF mutations in correlation with tumor depth, grade, regional and distant metastasis

Variable	n	BRAF positive n (%)	negative n (%)	p-level	difference test
T					
IS	4	0	4(2.84)		p = 0.88
2	12	1(9.09)	11(7.8)	Fisher's exact	*p = 0.01
3	96	3(27.27)	93(65.96)	test	p = 0.77
4a	23	2(18.18)	21(14.89)	**p=0.009	***p = 0.0002
4b	17	5(45.45)	12(8.51)		p = 0.6
G					
G1	5	0	5(3.55)	Fisher's exact	p = 0.52
G2	115	5(45.45)	110.(78.01)	test	*p = 0.015
G3	32	6(54.55)	26(18.44)	p=0.022	**p = 0.0047
N					
0	53	2(18.18)	51(36.17)		
1a	9	1(9.09)	8(5.67)		
1b	9	0	9(6.38)	Fisher's exact test	
1c	33	3(27.27)	30(21.28)	p=0.65	
2a	21	2(18.18)	19(13.48)		
2b	27	3(27.27)	24(17.02)		
M					
0	140	9(81.82)	131(92.91)	Fisher's exact test	
1c	5	2(1.18)	3(2.13)	p=0.069	
1a	7	0	7(4.96)		

*sig p<0.05, **sig p<0.01, ***sig p<0.0001

Table 3. BRAF mutations in correlation with tumor localization

Variable	n	BRAF positive n (%)	negative n (%)	p-level
Colon side				
right	35	6(54.55)	29(20.71)	X^2=6.55
left	116	5(45.45)	111(79.29)	p=0.01
Right				
coecum	18	2(33.33)	16(55.17)	Fisher's exact test
c.ascendens	13	4(66.67)	9(31.03)	p=0.28
c.transversum	4	0	4(13.79)	
Left				
sygma	31	0	31(27.93)	Fisher's exact test
rectum	70	4(80)	66(59.46)	p=0.4
c.descendens	15	1(20)	14(12.61)	

X^2 (Chi-square test)

differentiated tumors (45.45% *vs* 78.01%, p=0.015), while significantly more likely to have G3 category tumors, or poorly differentiated tumors (54.55% *vs* 18.44%, p=0.0047).

BRAF mutations were significantly associated with a higher disease stage (p=0.034). In the group with BRAF mutations, 10/11 tumors were diagnosed at stage III or IV, most commonly at stage IIIC (36.36%), followed by an identical percentage of tumors diagnosed at stage IIIB and IV (27.27%). In the group without BRAF mutations, the tumor was most commonly diagnosed at stage IIIB (36.17%), followed by a tumor at stage IIA (24.82%). In the individual stage comparisons, the incidence of stage IIA tumors in patients without BRAF mutations (24.82% *vs* 0%, p=0.049) and the incidence of stage IVC tumors in patients with mutations (27.27% *vs* 2.13%, p<0.0001) were confirmed as statistically significant (Table 4).

Table 4. BRAF mutations in correlation with stage of the disease

Variable	n	BRAF positive n (%)	negative n (%)	p-level	difference test
STAGE					
0	4	0	4(2.84)		p = 0.57
I	9	1(9.09)	8(5.67)		p = 0.64
II A	35	0	35(24.82)		*p = 0.049
II B	2	0	2(1.42)	Fisher's exact test *p=0.034	p = 0.69
II C	2	0	2(1.42)		p = 0.69
III A	1	0	1(0.71)		p = 0.78
III B	54	3(27.27)	51(36.17)		p = 0.55
III C	32	4(36.36)	28(19.86)		p = 0.2
IV A	7	0	7(4.96)		p = 0.45
IV C	6	3(27.27)	3(2.13)		p <0.0001
L					
no	20	2(18.18)	18(12.77)	Fisher's exact test p=0.64	
yes	132	9(81.82)	123(87.23)		
V					
no	55	3(27.27)	52(36.88)	Fisher's exact test p=0.75	
yes	97	8(72.73)	89(63.12)		

*sig p<0.05

PDL-1 expression

From 90 metastatic cases, we found positivity of PDL-1 in 17 cases. In this group of PDL1-positive tumors, more than 1% of positive tumor cells were detected in 10 tumors, more than 10% in 4 tumors, more than 50% in 3 tumors (Figure 1). All 3 cases with >50% cut off showed right-sided localization and all had BRAF mutations.

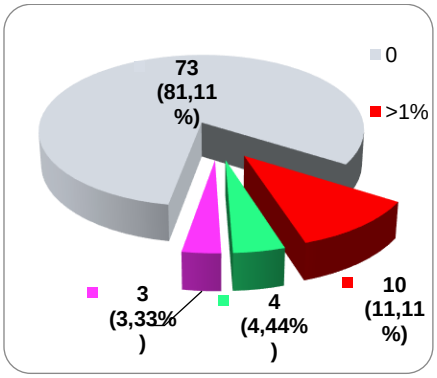

Fig. 1. PDL-1 expression by cut-offs

Discussion

Molecular profiling is an essential component in the personalized treatment of metastatic colorectal carcinoma. BRAF gene mutation is one of the alterations that has made a large impact over the past decade, having a strong implication in the prediction of the response of therapeutic regimens[1,7-9]. By analyzing PD-L1 expression levels in tumor tissues, researchers have found that high PD-L1 levels are often associated with worse prognosis and a higher risk of disease recurrence in patients with rectal carcinoma. By identifying PD-L1 expression as a prognostic marker in rectal carcinoma, clinicians can better stratify patients into different risk groups, allowing for more personalized treatment decisions and improved patient outcomes. Additionally, targeting PD-L1 expression could also be a potential therapeutic strategy in rectal carcinoma, as blocking the PD-1/PD-L1 pathway can potentially enhance the patient's immune response against the tumor and improve treatment efficacy.

However, the use of PD-L1 expression as a prognostic marker in rectal carcinoma is still a topic of debate within the medical community[9,10].

The study by Noh *et al.* suggested that other factors, such as tumor mutational burden and immune cell infiltration, may play a more critical role in determining patient outcomes[11]. Additionally, the dynamic nature of PD-L1 expression and its interaction with the tumor microenvironment make it a complex marker to rely on for prognostic predictions.

Furthermore, the use of PD-L1 expression as a predictive biomarker for immunotherapy in colorectal carcinoma is not yet well-established. Clinical trials evaluating the efficacy of immune checkpoint inhibitors in this patient population have not consistently shown a clear benefit based on PD-L1 expression levels[11]. In our study, only 3 patients showed positivity in more than 50% of tumor cells.

In light of these conflicting findings, further research is necessary to fully understand the role of PD-L1 expression as a prognostic marker in colorectal carcinoma and its potential implications for treatment decisions. While it holds promise, its clinical utility and reliability as a prognostic tool requires rigorous evaluation before widespread integration into patient management[12-15].

In parallel, ongoing clinical trials investigating the efficacy of immune checkpoint inhibitors in colorectal carcinoma patients are anticipated to provide valuable insights into the utility of PD-L1 expression as a predictive biomarker for immunotherapy[16-19]. These findings have the potential to revolutionize the treatment landscape for colorectal carcinoma and pave the way for more personalized and effective management strategies[20-23].

Conclusion

In conclusion, the association between BRAF mutation and PD-L1 expression represents a novel and clinically relevant aspect of CRC biology. BRAF mutations in CRC are associated with aggressive tumor characteristics, including greater depth, high grade, and right-sided location. Understanding the molecular crosstalk between these pathways not only sheds light on the underlying mechanisms of tumor immune evasion but also paves the way for the development of personalized therapeutic approaches leveraging both targeted agents and immunotherapy to improve outcomes for patients with BRAF-mutated CRCs.

PD-L1 expression in rectal carcinoma holds great potential as both a prognostic marker and a therapeutic target. Future research should focus on validating the utility of PD-L1 expression as a prognostic marker and therapeutic target in colorectal carcinoma.

Conflict of interest statement. None declared.

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