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Immunohistochemical expression of p53, beta-catenin, and PD-L1 in colorectal carcinoma with respect to grade and stage-an observational study in a tertiary care center

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ABSTRACT

Background: Colorectal cancer (CRC) has consistently shown a global rise in incidence. p53 exerts regulatory function in signaling processes, with the p53 mutation accelerating the late stage of colorectal carcinogenesis. Programmed death ligand 1 (PD-L1) is an immune checkpoint regulator whose role in targeted therapy of CRCs needs to be evaluated. Beta-catenin plays a fundamental role in cell adhesion, with long-term effects on disease progression and survival. A detailed evaluation of all these factors is essential to tailoring new therapeutic approaches in advanced-stage cases. **Aims and Objectives:** To study the immunohistochemical (IHC) expression of p53, PD-L1, and beta-catenin in different histologic grades and stages of colorectal carcinomas. **Materials and Methods:** A total of 29 cases of segmental colectomy were included in the study population. Tumor sections were sent for IHC study of p53, PD-L1, and beta-catenin and interpreted as per standard protocol. Findings were tabulated against the histologic grade and stages of CRCs. Statistical analysis was done using SPSS 25.0 to test for statistical significance at $P<0.05$. **Results:** The study population predominantly consisted of males (66%), mainly in the age group of 46–60 years. p53 was significantly more expressed in left colonic tumors. PD-L1 expression was also significantly associated with histologic grade and nodal metastasis. High beta-catenin expression was seen in well-differentiated adenocarcinomas and lower T and N-stage disease. Positive p53 also correlated with positive PD-L1 expression. However, expression of p53 was also high in tumors with low beta-catenin expression. **Conclusion:** p53, PD-L1, and beta-catenin may have a role in prognostication and predicting long-term survival outcomes in CRC cases. These may also act as potential therapeutic targets with a more detailed evaluation and a larger sample size of CRC cases.

Key words: Beta-catenin; Histologic grade; p53; Programmed death ligand 1; Stage

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INTRODUCTION

Colorectal cancer (CRC) is the third most common cancer in men and the second most common cancer in women worldwide.[1] CRC becomes a serious problem for healthcare in Asian countries too, such as China, Japan, South Korea, and Singapore, with a 2–4 fold increase in the incidence during the last decades.[2] There is a consistent rise in the incidence of colon cancer across all Indian cancer registries, ranging from 20% to 124%/year.[3] So, more efficacious approaches are urgently needed for CRC patients. Surgery still remains the mainstay for treatment, but more potent adjuvant therapy may augment the chance of cure in high-risk cases.[4]

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p53 is a stress-inducible transcription factor that exerts regulative functions in multiple signaling processes. p53 mutation occurs in approximately 40–50% of sporadic CRC.[5] The status of the p53 mutation is closely related to the progression and outcome of sporadic CRC. The missense-type p53 mutation, together with the loss of wild-type p53, may accelerate the late stage of colorectal carcinomas with the activation of oncogenic and inflammatory pathways.[6]

The discovery of immune checkpoint inhibitors has revolutionized cancer treatment regimes, and checkpoint inhibitors have already become part of the therapeutic standard for different human cancer types.[7] programmed death ligand 1 (PD-L1) is one such regulator whose potential role in CRC needs to be evaluated, with definite therapeutic potential.

Beta-catenin plays a fundamental role in the regulation of the E-cadherin-catenin cell adhesion complex, which plays an important role in CRC. Mutations in either the APC or beta-catenin genes in colorectal cancer cells result in up-regulation of protein expression and subsequent cytoplasmic and nuclear distribution of beta-catenin.[8] Because of its significant role in cell-cell interaction, beta-catenin mutations may affect epithelial-mesenchymal transition and metastasis, with long-term effects on disease progression and survival.[9]

This study aims to assess the expressions of p53, beta-catenin and PD-L1 in varying grades and stages of CRCs and to assess any possible cross-talks among them in cancer progression and survival.

Aims and objectives

1. To study the immunohistochemical (IHC) expressions of p53 in different grades and stages of colorectal carcinoma
2. To study the IHC expressions of beta-catenin in different grades and stages of colorectal carcinoma
3. To study the IHC expressions of PD-L1 in different grades and stages of colorectal carcinoma
4. To study for any correlation among expressions of p53, beta-catenin, and PD-L1 in different CRC cases.

MATERIALS AND METHODS

This cross-sectional, observational study was conducted in a tertiary care center in north-east India after approval from the Institutional Ethical Committee (reference number: ECR/322/Inst/WB/2013/RR-20). The inclusion criteria were all CRC cases with well-defined growth on gross examinations sent to the department of pathology at R. G. Kar Medical College for histopathological study during the study period who had given written consent for participation in the study. All those cases that have received neoadjuvant chemotherapy with a complete or near-complete response and show no well-defined mass on gross examination of the specimen, cases with histopathological diagnosis other than carcinomas (e.g., lymphomas, GIST, sarcomas, etc.), and those who did not wish to participate were excluded from the study. Finally, the study consisted of a cohort of 29 patients, all of whom have undergone segmental colectomy in the institute, and specimens were sent for histopathological evaluation in the Department of Pathology. Formalin-fixed, paraffin-embedded sections were stained with routine Hematoxylin and Eosin stain; histologic type, World Health Organization (WHO) grading, staging, and nodal status were evaluated and recorded. Blocks suitable for IHC analysis of p53, beta-catenin and PD-L1 were selected.

IHC analysis was done by the peroxidase and anti-peroxidase methods using the following antibodies: p53 (Mouse Monoclonal IHC Antibody, BP-53-12; PathnSitu), beta-catenin (Rabbit Monoclonal Antibody, EP35; PathnSitu), and PD-L1 (Rabbit Monoclonal Antibody, B7H1P; PathnSitu). The chromogen used was 3,3'-diaminobenzidine, counterstained with Harris hematoxylin. Specificities of anti-p53 were confirmed using breast carcinoma sections as positive controls with known expression data. Colon carcinoma cases itself served as a positive control for beta-catenin, whereas placental tissue was the positive control for PD-L1[10] (Figure 1).

The stained IHC slides were examined under a light microscope. p53 staining was reported qualitatively as positive or negative, where nuclear staining in >10% of tumor cells was considered positive as per the study conducted by Kay et al.[11] The scoring of beta-catenin stain was as per the criteria proposed by Allred et al.[12] The staining intensity was estimated on a four-step scale (0=none, 1=weak, 2=moderate, and 3=strong), whereas the fraction of the stained cells was scored as: score 0: ≤10%, score 1: 11–33%, score 2: 33–66%, and score 3: ≥67% positive cancer cells. The final staining score was assigned based on the multiplication of the staining intensity as well as the percentage of the positive cells and graded as follows: 0=0; 1=1–3, 2=4–6, and 3=7–9; low expression = 0 or 1; and high expression =2 or 3.[13] PD-L1 staining was reported in a qualitative manner as positive when ≥5% membranous positive staining of any intensity was noted in tumor cells.[14]

All the data were tabulated in a master sheet and analyzed using IBM SPSS Version 25.0 software. Descriptive

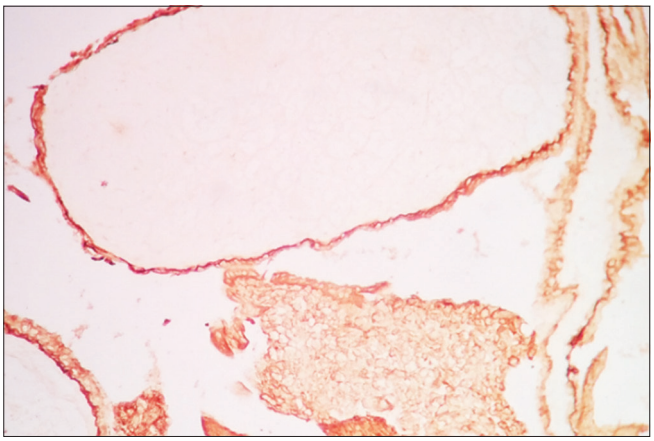

Figure 1: Placental tissue as positive control for PD-L1 immunohistochemistry (×40 magnification)

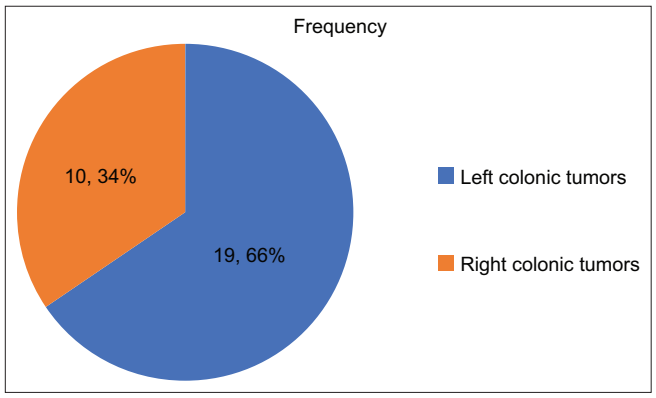

Figure 2: Pie chart showing distribution of left and right colonic tumors

statistics like mean, median, and mode were used wherever applicable. Pearson chi square (χ^2 test) and Fisher exact tests were used to calculate statistical significance, where the P-value is considered <0.05.

RESULTS

The study population predominantly consisted of males (male=19; female=10). The age range was 42–71 years, the mean being 57 years. The majority of the population belonged to the age group of 46–60 years (55%). The demographic characteristics have been summarized in the table below (Table 1).

Table 1: Distribution of study population based on age group and gender

Demographic characteristics	Frequency (%)
Gender	
Male	19 (65.5)
Female	10 (34.5)
Age group (years)	
≤45	3 (10.3)
46–60	16 (55.2)
61–75	10 (34.5)

The proportion of left colonic tumors is higher than that of right colonic tumors (Figure 2).

The major histologic type of colonic tumors were adenocarcinomas (27, 93.1%), with only 2 cases of mucinous carcinomas (2, 6.9%). The majority of the histologic grade of the adenocarcinomas was WHO Grade 2 (17, 63%) followed by Grade 1 (7, 26%) and Grade 3 (3, 11%). Two cases of mucinous carcinomas have been excluded from the WHO histologic grading system, which is applicable to conventional adenocarcinomas only. In the pathologic staging of the tumor, the majority belonged to pT3 (16, 55.2%), followed by pT2 (10, 34.5%) and pT4 (3, 10.3%). As for the nodal staging, a major proportion belonged to the N_0 stage (12, 48%), followed by the N_1 stage (10, 40%), and N_2 (3, 12%). Four cases have been excluded from nodal staging as they belonged to the N_X (nodal stage undetermined) category. On evaluation of IHC-stained sections, 19 cases were p53 positive (66%) and 10 cases were p53 negative (34%). 16 cases were PD-L1 positive (55.2%) and 13 cases were PD-L1 negative (44.8%). Out of the total number of cases, 21 showed high beta-catenin expression (72.4%) and 8 showed low expression (27.6%). All the above observations have been depicted in the table below (Table 2).

On performing the chi-square test, p53 was seen to be significantly expressed in left colonic tumors (P=0.0002) (Figure 3). p53 was also significantly expressed more in PD-L1-positive tumors (P=0.04799). However, p53 was significantly expressed in tumors with low beta-catenin expression (P=0.03). However, no statistical association was found between p53 expression and the histologic grade, T stage, or N stage of the tumors. As was PD-L1, it was seen to be significantly expressed in higher grades of the tumor (P=0.0004) (Figure 4) and a higher nodal stage of the disease (P=0.0003). No significant association was seen with PD-L1 expression, tumor laterality, T staging, or concurrent beta-catenin expression. Beta-catenin, on the other hand, is seen to be expressed significantly higher with a lower histologic grade (Figures 5 and 6), a lower T stage, and a lower nodal stage of the disease (P<0.05). However, no association was seen between beta-catenin expression and the laterality of the tumors. All these findings are summarized below (Table 3).

DISCUSSION

CRC, being one of the most common cancers, has shown 1.9 million new cases in the year 2020 and is responsible

for about 935,000 cancer-related deaths worldwide.[15] There is a rising global incidence of CRC cases, currently comprising 11% of all cancer diagnoses.[16] The male population was seen to be more commonly affected than females in this study population, concurrent with the findings by Rawla et al.[17] The majority of the study population belonged to the age group of 46–60 years, with the mean age being 57 years. This was in contrast with the trend in the western population that has shown a rising incidence among the age group of 20–49 years, and incidence beyond 50 years has shown a steady decrease.[18] This difference in trends amongst the western and Indian

Table 2: Distribution of study population based on laterality, histologic type, grade, T staging, Nodal staging and immunohistochemical staining patterns of p53, PD-L1 and Beta-catenin

Parameters	n (%)
Laterality of tumors (n=29)	
Left	19 (65.5)
Right	10 (34.5)
Histologic type (n=29)	
Adenocarcinoma	27 (93.1)
Mucinous carcinoma	2 (6.9)
Histologic grade (n=27)	
Grade 1	7 (26)
Grade 2	17 (63)
Grade 3	3 (11)
pT staging (n=29)	
T1	0
T2	10 (34.5)
T3	16 (55.2)
T4	3 (10.3)
Nodal staging (n=25)	
N0	12 (48)
N1	10 (40)
N2	3 (12)
p53 stain (n=29)	
Positive	19 (66)
Negative	10 (34)
PD-L1 (n=29)	
Positive	16 (55.2)
Negative	13 (44.8)
Beta-catenin (n=29)	
High expression	21 (72.4)
Low expression	8 (27.6)

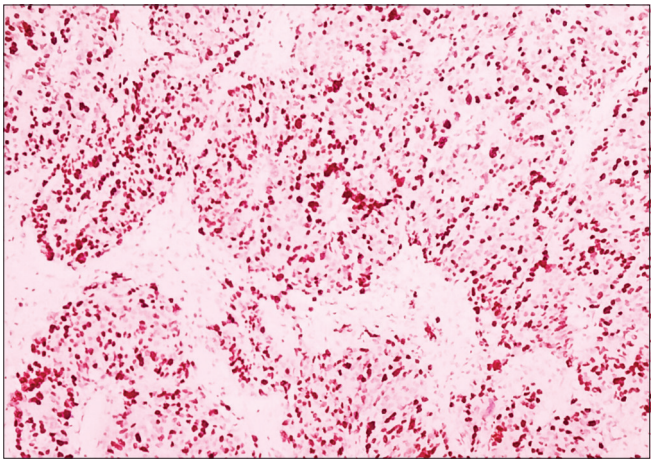

Figure 3: p53 positivity in adenocarcinoma of descending colon (IHC, ×100 magnification)

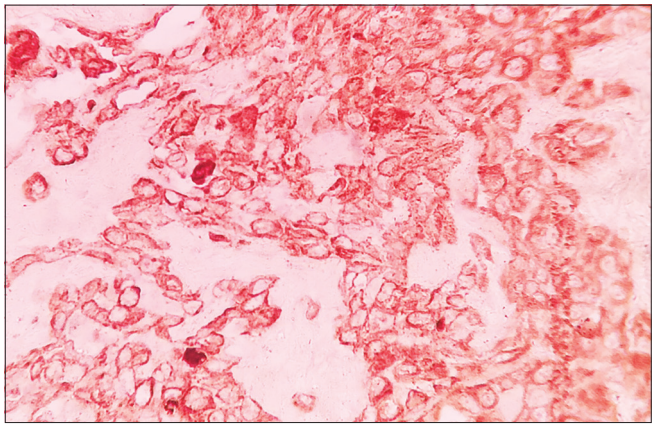

Figure 4: Positive PD-L1 staining in grade 3 adenocarcinoma (IHC, ×400 magnification)

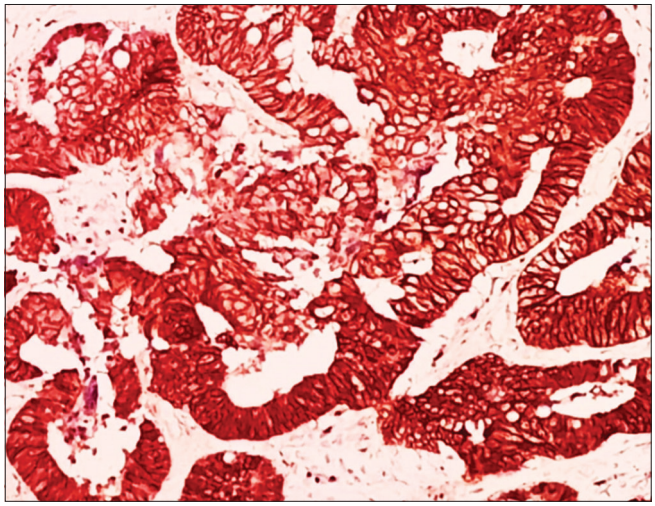

Figure 5: Strong cytoplasmic and nuclear stain for beta-catenin in grade 2 adenocarcinoma (IHC, ×100 magnification)

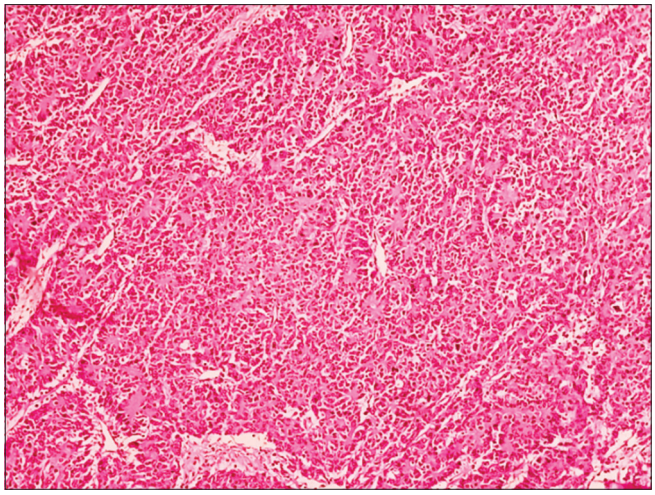

Figure 6: Loss of beta-catenin expression in poorly differentiated adenocarcinoma (IHC, ×100 magnification)

Table 3: Correlation between immunohistochemical staining patterns of p53, PD-L1 and Beta-catenin with laterality, histologic grade, T and N staging of colorectal carcinoma cases

Parameters	Positive p53	Negative p53	P (significant if<0.05)	Remarks
Laterality of tumors				
Right	2	8	0.0002	Significant (Chi-square test)
Left	17	2		
Histologic grade				
Grade 1	2	5	0.085	Not significant (Fisher's exact test)
Grade 2	12	5		
Grade 3	3	0		
T staging				
T2	7	3	0.41	Not significant (Fisher's exact test)
T3	9	7		
T4	3	0		
Nodal staging				
N_0	7	5	0.51	Not significant (Fisher's exact test)
N_1	7	3		
N_2	3	0		
PD-L1 expression				
Positive	13	3	0.04799	Significant (Chi-square test)
Negative	6	7		
Beta-catenin expression				
High	11	10	0.0265	Significant (Chi-square test)
Low	8	0		
Parameters	**Positive PD-L1**	**Negative PD-L1**	**P (significant if<0.05)**	**Remarks**
Laterality of tumors				
Right	3	7	0.48	Not significant (Fisher's exact test)
Left	13	6		
Histologic grade				
Grade 1	0	7	0.0004	Significant (Fisher's exact test)
Grade 2	13	4		
Grade 3	3	0		
T staging				
T2	3	7	0.0721	Not significant (Fisher's exact test)
T3	10	6		
T4	3	0		
Nodal staging				
N_0	3	9	0.0003	Significant (Fisher's exact test)
N_1	10	0		
N_2	3	0		
Beta-catenin expression				
High	10	11	0.185	Not significant (Chi-square test)
Low	6	2		
Parameters	**High beta-catenin expression**	**Low beta-catenin expression**	**P (significant if<0.05)**	**Remarks**
Laterality of tumors				
Right	8	2	0.51	Not significant (Chi-square test)
Left	13	6		
Histologic grade				
Grade 1	7	0	0.005	Significant (Fisher's exact test)
Grade 2	14	3		
Grade 3	0	3		
T staging				
T2	10	0	0.002	Significant (Fisher's exact test)
T3	11	5		
T4	0	3		
Nodal staging				
N_0	10	2	0.03	Significant (Fisher's exact test)
N_1	7	3		
N_2	0	3		

subcontinents may be attributable to the stark contrast in lifestyles between these 2 groups of people, with obesity, smoking, and alcoholism dominating the lives of developed nations in conjunction with sedentary lifestyles.

Left colonic tumors were more predominant in this study group, with a reported incidence of 65.5%, which was concurrent with findings by Yang et al.[19] Right and left colonic tumors are two separate entities based on their embryology, pathology, and prognosis.[20] Therefore, an attempt has been made to find any significant association between laterality and differential expressions of p53, PD-L1, and beta-catenin.

A major proportion of cases in this study showed positive p53 expression (66%), which was synchronous with the findings by Wang et al.[21] left colorectal carcinomas showed a significantly positive p53 expression, as seen in this study setting. A concurrent association between p53 expression and distal colon tumors was also observed by Scott et al.[22] However, Wang et al. showed no significant association between the site of neoplasm and p53 expression. p53 was not seen to be significantly associated with the histologic grade, T, and N staging of the tumor. Contrasting observations were however seen by Wang et al., which showed higher p53 expression with a poor degree of differentiation and a higher T and N stage of the disease.[21] This disparity can be attributed to the small sample size in this study population, which may not be representative of the tumor biology of these cases. p53 plays an important role in regulating the cell cycle, DNA damage repair, regulating immune responses, and so on.[23] Hence, any mutation in the p53 gene is seen to play a major role in the grade, metastatic potential, staging, and overall disease-free survival of a neoplasm. The p53 mutation may also act as an independent prognostic factor in colon cancer, with a major pathophysiologic role in the adenoma-carcinoma sequence.[24] Hence, with further studies, p53 may be utilized to tailor precise treatment options for CRC cases with poor prognosis at molecular levels.

Of all cases, 55.2% were PD-L1 positive, in contrast to 28% positivity in a study conducted by Elfishawy et al.[14] This study could demonstrate no statistical significance between PD-L1 expression and the laterality of tumors, which was, however, in stark contrast with the findings by Inaguma et al.[25] and Valentini et al.[26] that showed high PD-L1 expression in right colonic tumors. PD-L1 expression was also seen to be statistically associated with higher histologic grades of the tumor and a higher nodal stage of the disease. However, no significant association was seen between PD-L1 expression and T staging of the tumor. The correlation with the histologic grade of the tumor was also shown by Kim et al.,[27] Inaguma et al.,[25] Valentini et al.[26] and Zhong et al.[28] where PD-L1 showed 100% expression in poorer grades of differentiation. In separate studies by Masugi et al.[29] and Wang et al.,[30] no such correlation could be established. Similar to our study, no statistical significance was found with T stage and PD-L1 expression by Wang et al.,[30] Rosenbaum et al.,[31] Masugi et al.,[29] Enkhbat et al.,[32] and Zhong et al.[28] Concurrent to this study, El Jabbour et al.[33] could demonstrate a positive statistical association between nodal stage and PD-L1 expression in CRC cases. Masugi et al.,[29] and Zhong et al.,[28] however, could not establish any statistical significance with the nodal stage of the disease. The PD-1 pathway is a major immune response checkpoint and has been utilized by many solid tumors, including CRC, to generate an immunosuppressive tumor microenvironment, avoid immunologic surveillance, and promote cancer growth.[34] These make PD-L1 a promising target for cancer immunotherapy, and its role in colorectal cases may be further evaluated and authenticated with a larger study population to treat cases with adverse prognostic outcomes.

A major population of 72.4% had high expression of beta-catenin, which was similar to the findings by Tunuguntla et al.[35] In this study setting, high beta-catenin expression was seen to be significantly associated with a low histologic grade, a lower T stage, and a lower nodal stage of the disease. This may imply a loss of beta-catenin expression with a higher histologic grade, higher T staging, and widespread nodal metastasis, which indicates an aggressive and advanced disease stage. This corroborated with the findings by Gomaa et al.[36] Contrasting observations were seen in studies by Choi et al.,[37] and Tunuguntla et al.,[35] where higher beta-catenin expression was associated with advanced TNM staging and the histologic grade of the disease. Beta-catenin is under the functioning of intracellular Wnt signaling pathways, with mutations in APC, beta-catenin, or any genes of Wnt signaling pathways leading to overaccumulation of beta-catenin protein intracellularly, followed by its nuclear translocation. Intranuclear beta-catenin stimulates various other activities, leading to cell proliferation and tumorigenesis.[38] Beta-catenin plays a significant role in the adenoma-carcinoma sequence, with subsequent targets for chemotherapy or prognostication with long-term disease-free survival. This particular property of beta-catenin may be explored through a larger study population and a larger sample size in order to draw any conclusive opinion.

In this study population, p53 expression was seen to be higher in PD-L1-positive tumors, with a significant statistical association. There is a paucity of literature that has studied both of these attributes in colorectal carcinoma cases. However, in a study by Agersborg et al.,[39] no significant association was seen between PD-L1 and p53 expression in CRC cases. p53 was also significantly expressed more in tumors with low beta-catenin

expression in this study population. In the initial stages of CRCs, there is an overexpression of beta-catenin that promotes a functional increase in p53 that would restrict tumor proliferation by feedback mechanism.[40] However, in late stages, there may be a mutation in p53 with a block in this basic feedback mechanism, with aberrant nuclear accumulation of beta-catenin and tumor progression.[41] No statistically significant association was observed between PD-L1 and beta-catenin expression in this setting. In a study by Fu et al.,[42] it was observed that PD-L1 interacts to increase beta-catenin signaling and beta-catenin-targeted gene expression and may form a positive feedback loop to promote cancer progression through cancer stem cell maintenance and expansion. Thus, a larger study population may be considered to explore this domain of tumorigenesis.

Limitations of this study

The study is limited by the smaller sample size and hospital-based study setting. Further studies with a community-based approach and a larger sample size may help in extrapolating the findings and be a better representative of the heterogenous nature of the CRC cases.

CONCLUSION

Colorectal carcinomas are showing a global increase in incidence across the world. This has compelled researchers to identify important prognostic indicators for advanced and treatment-resistant cases of CRCs. In this study population, p53 was seen to be significantly expressed more in left colonic tumors. PD-L1 expression was also significantly associated with histologic grade and nodal metastasis. High beta-catenin expression was seen in well-differentiated adenocarcinomas, lower T and N stage disease, with subsequent loss of expression in progressive and advanced stages. Positive p53 was also correlated with positive PD-L1 expression. However, expression of p53 was high in tumors with low beta-catenin expression. All these may indicate a possible interplay and molecular feedback mechanism between different pathways that may regulate the cell cycle and tumor progression. This, in turn, may pave the way for potential therapeutic targets for advanced and treatment-resistant cases of colon cancer.

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