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A Study of Expression of SATB2 Protein In Colorectal Adenocarcinoma And Its Correlation With Clinicopathological Parameters

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ABSTRACT

Background: Colorectal cancer is the third most common cancer in the world and second most common in terms of mortality. Disease progression is associated with tumour invasion and metastasis; therefore, it is essential to recognise the associated protein involved in tumour progression. SATB2 (Special AT-rich Sequence-Binding Protein 2) is a highly specific marker for colorectal tissue. Decreased expression of SATB2 is associated with poor prognosis. This study aimed to evaluate the histomorphology characteristics, immunohistochemical expression of SATB2, and its correlation with clinicopathological parameters in colorectal adenocarcinoma.

Materials & Methods: The cross-sectional study was conducted in the Department of Pathology, MMCH&RI, Kanchipuram, India, in the period between October - 2023 to May 2024. 60 cases of colorectal adenocarcinoma were included in the study. Formalin-fixed, paraffin-embedded tissue sections were subjected to immunohistochemical staining using SATB2-specific antibodies. Data analysis was done using software-statistical package for social sciences (SPSS, version 26).

Results: Among the total 60 specimens, SATB2 expression was observed in 65% of cases, more than half 55%(n=33) were male and 65% (n= 39) were left side tumours. G3 tumors are more likely to be SATB2 negative. Majority of cases with reduced SATB2 expression show lymphovascular invasion and regional lymph node metastasis.

Conclusion: The results of this study demonstrates that diminished expression of SATB2 is correlated with regional lymph node metastasis, lymphovascular invasion and increased tumor grading, all of which serve as indicators of unfavorable prognosis. These findings help clinicians in stratifying patient risk and facilitate treatment approaches, such as incorporating adjuvant chemotherapy.

Keywords: *SATB2, Adenocarcinoma, Invasion, Metastasis*

1. INTRODUCTION

Colorectal cancers (CRC) represent the third most prevalent form of malignancy on a global scale and rank second with regard to mortality rates.[1,2] This category of cancer constitutes approximately 10% of all diagnosed cancers and serves as the second predominant contributor to disability-adjusted life years (DALYs) attributable to cancer on an international level.[3,4] Moreover, Colorectal cancer is the second leading cause of cancer related fatalities overall and the top cause of cancer death in men younger than 50 years of age.[5] The prevalence of colorectal malignancies in India is demonstrating a rising trajectory. According to data from the cancer registry initiative, the incidence rate stands at 8.5%. In recent years, the diagnostic landscape for colorectal cancers has undergone significant transformations, encompassing advancements in genetic research as well as improvements in molecular and imaging methodologies.[6] In the context of our healthcare system, the identification of colorectal malignancies tends to occur at a later stage, consequently resulting in a generally

suboptimal therapeutic outcome. The etiology of colorectal cancer is frequently linked to the benign proliferation of mucosal epithelial cells. Polyps are non-cancerous neoplasms that may progress insidiously over a period of 10 to 20 years prior to undergoing malignant transformation. The predominant variant is the adenoma, or polyp that arises from glandular epithelial cells, which are responsible for the secretion of mucus that lines the colon. Despite the correlation between polyp size and the heightened risk of neoplastic progression, it is notable that only approximately 10% of all adenomatous polyps evolve into aggressive forms of malignancy. Adenocarcinoma, a form of invasive cancer, originates from these polyps and constitutes 96% of all colorectal carcinomas.[7]

SATB2 (The special AT-rich sequence-binding protein 2) represents a novel DNA-binding protein and nuclear transcription factor comprising 733 amino acids in length. SATB2 is implicated in the processes of gene transcription and chromatin remodeling.[8] This protein exhibits a distinctive expression pattern, localized within the nuclei of various cell types. Under normal physiological conditions, SATB2 is expressed in the brain, regions of bone formation, the gastrointestinal tract, colon, kidneys and lymphoid tissues.[9] Immunohistochemical analyses have demonstrated that SATB2 is prominently expressed not only in normal and neoplastic osteoblastic tissues but also in the normal epithelial cells of the colorectal and appendiceal regions. Later studies showed that SATB2 is a protein with high tissue specificity, mainly found in the glandular cells of the lower gastrointestinal tract. This assertion is substantiated by an array of empirical investigations demonstrating that SATB2 serves as a valuable and specific diagnostic biomarker for distinguishing tumors of colorectal origin from those arising from other sites, including ovarian and pancreatic neoplasms.[10] In addition to its role as a diagnostic biomarker, SATB2 has been identified as having prognostic implications in colorectal carcinoma, as evidenced by several additional studies.[11] Colorectal cancer progression, marked by tumour invasion and metastasis, significantly affects patient outcomes. Therefore, understanding the roles of genes and their protein products involved in this progression is crucial. Lower SATB2 expression has been linked to metastasis and worse prognosis in colorectal cancer, suggesting that SATB2 loss may contribute to disease advancement.[12] The present investigations were undertaken to assess the expression levels of SATB2 in colorectal adenocarcinoma and to explore its relationship with various clinicopathological parameters.

2. MATERIALS AND METHODS

The cross-sectional study was conducted in the Department of Pathology, MMCH&RI situated in Kanchipuram district of Tamil Nadu, South India. Scientific and Ethical committee approval was obtained and informed consents from the patients was exempted by Institutional Ethics Committee approval (Ethical approval number: MMCH & RI IEC/ PG/ 38/ OCT/ 23). The study period was eight months between October 2023 to May 2024. This study included 60 colorectal adenocarcinoma cases. The study population consisted of primary colorectal adenocarcinoma cases of both resected surgical specimens and small biopsies. All colectomy specimens, including right and left hemicolectomy, low anterior resection, abdominoperineal resection, and total colectomy specimens, were eligible for inclusion. The specimen of patients with a history of neoadjuvant chemotherapy and radiotherapy, inadequate tissue material on paraffin embedded blocks and non-neoplastic colorectal lesions were excluded from the study.

Study procedure

Clinicopathologic data was collected after reviewing histopathological registers and cancer registers in the Department of Pathology, MMCH&RI, Kanchipuram. Demographic details, Side affected, Tumour grading as Well differentiated(G1), Moderately differentiated(G2) and Poorly differentiated(G3), TNM Staging, Lymphovascular invasion, Perineural invasion, Lymph node metastasis and SATB2 expressions were noted.

Immunohistochemistry (IHC)

Sections of tissue (4-5 microns thick) were cut using a rotary microtome (Leica: RM2255) and placed on positively charged slides. Antigen retrieval on tissue sections was performed by microwave method (Prestige PMDG20PC). IHC was performed with Peroxidase-Antiperoxidase system, staining was done using primary antibody (rabbit monoclonal; clone EP281) and secondary antibody (Poly-excel Horse Radish Peroxidase/ DAB 3,3’ Diaminobenzidine).

Grading of IHC expression

SATB2 expression in colorectal adenocarcinoma samples was assessed via immunohistochemical (IHC) staining, following the scoring system. The staining was evaluated semi-quantitatively as a sum of extent and intensity of nuclear staining as follows,

Extent of Nuclear Staining: It’s a measure of the proportion of cells expressing the SATB2 protein.

- 0 - 1% (Virtually no staining or very weakly positive) - 0
- 2 - 25% (A small percentage of cells are positive) - 1
- 26 - 75% (A significant portion of cells are positive) - 2
- >75% (The majority of cells show positive staining) - 3

Intensity of Nuclear Staining: It's a measure of staining level of SATB2 protein within those cells.

- Negative (No visible staining) - 0
- Weak (Faint staining) - 1
- Moderate (Visible staining, but not very intense) - 2
- Strong (Intense, dark staining) - 3

A composite score of 3 or higher was considered positive for SATB2 expression, while a score below 3 was considered negative for statistical analysis, based on the previous study by Wang S et al.[12] The collected data was then entered into Microsoft Excel and subsequently analysed using Statistical Package for Social Sciences (SPSS) software version 26. A p-value of less than 0.05, obtained from Chi-square tests, was considered statistically significant when assessing the relationship between SATB2 expression and clinicopathological features.

3. RESULTS

A total of 60 specimens of colorectal adenocarcinoma were included in our study. The overall prevalence of SATB2 expression was 65%. Among the total 60 specimens, 40 were resected and 20 specimens were biopsy. Most of the specimens (87.4%) belonged to age of more than 50 years. More than half (55%) of the specimens were male origin. About 65 % of the specimens were obtained from left side.

Table 1: SATB2 expression as per age, gender, side of specimens and tumor grading obtained from the study subjects (N=60)

Variables	SATB2 Positive (n=39)	SATB2 Negative (n=21)	Total (n=60)	P value
Age				
<50	5(12.8%)	5(23.8%)	10 (16.6%)	0.27
>50	34(87.2%)	16(76.2%)	50(87.4%)	
Gender				
Male	18(46.1%)	15(71.4%)	33 (55%)	0.01*
Female	21(53.9%)	6(28.6%)	27(45%)	
Side				
Right	15(38.5%)	6(28.6%)	21(35%)	0.44
Left	24(61.5%)	15(71.4%)	39(65%)	
Tumor differentiation/Grading(G)				
Well-differentiated (G1)	30(76.9%)	8 (38%)	38(63.3%)	0.003*
Moderately differentiated(G2)	9(23.1%)	10(47.6%)	19(31.6%)	
Poorly differentiated(G3)	0	3(14.4%)	3(5.1%)	

As shown in Table 1, a slightly higher percentage of patients >50 years old had SATB2 negative tumors (76.2%) compared to those <50 years of age (23.8%) showing no statistically significant association between age and SATB2 expression. Males showed a higher percentage of SATB2 negative tumors (71.4%) compared to females (28.6%) showing a significant association between gender and SATB2 expression. In addition, the expression of SATB2 decreases with increase in tumor grading. G1 tumors (Figure 1) predominantly show SATB2 positivity (76.9%), while G3 tumors (Figure 3) are more likely to be SATB2 negative. The above findings are statistically significant at p value <0.05.

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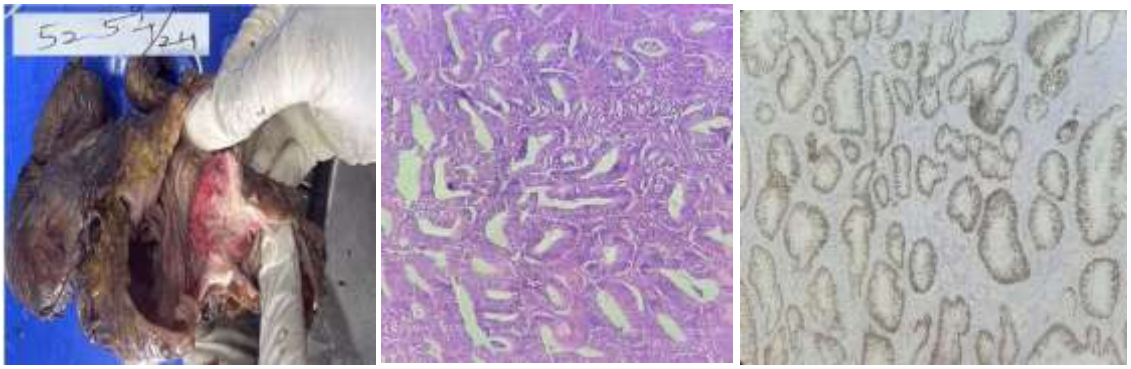

Figure 1: Well-differentiated adenocarcinoma. (a) Macroscopic view of an ulceroproliferative growth in the descending colon; (b) Microscopic image (10x magnification, Hematoxylin & Eosin stain) of the tumor, showing glandular-patterned cells; and (c) Immunohistochemical stain for SATB2 (10x magnification) with a score of 6, indicating strong staining in over 75% of the cells.

Table 2: Association of SATB2 expression with Final Staging of resected specimens of study subjects (N=40)

Final staging	SATB2 Positive (n=25)	SATB2 Negative (n=15)	Total (n=40)	P value
T1	5(20%)	5(33.3%)	10(25%)	0.67
T2	8(32%)	5(33.3%)	13(32.5%)	
T3	11(44%)	5(33.3%)	16(40%)	
T4	1(4%)	0	1(2.5%)	

With regards to final staging (Table 2) done among the resected 40 specimens, it has been found that the association of SATB2 expression with final tumor staging is not statistically significant.

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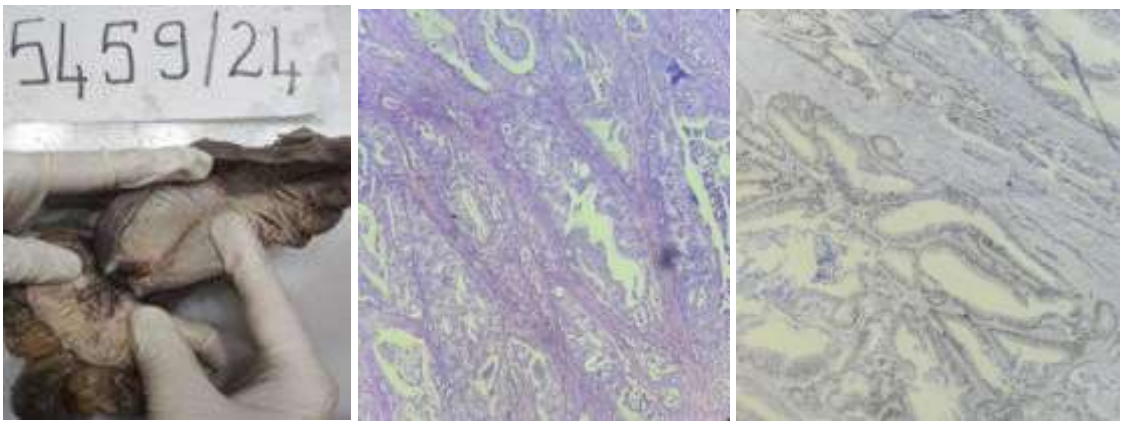

Figure 2: Moderately differentiated adenocarcinoma. (a) Macroscopic view reveals a napkin-ring constriction in the rectum. (b) Microscopic image (10x magnification, Hematoxylin & Eosin stain), the tumour cells appear in glandular and nested arrangements. (c) Immunohistochemical staining for SATB2 (10x magnification) showing weak staining in approximately 50% of the cells, corresponding to a score of 3.

Table 3: Association of SATB2 expression with lymphovascular & perineural invasion of resected specimens of study subjects (N=40)

Variables	SATB2 Positive (n=25)	SATB2 Negative (n=15)	Total (n=40)	P value
Lymphovascular invasion				
Yes	8(32%)	12(80%)	20(50%)	0.03*
No	17(68%)	3(20%)	20(50%)	
Perineural invasion				
Yes			7(17.5%)	
No	3(12%)	4(26.7%)	33(82.5%)	
	22(88%)	11(73.3%)		0.23

In the mentioned Table 3, it was determined that the expression of SAT2 was notably diminished in specimens exhibiting lymphovascular invasion (32%) in contrast to specimens lacking lymphovascular invasion (68%), which is statistically significant but there were no significant differences with perineural invasion was identified.

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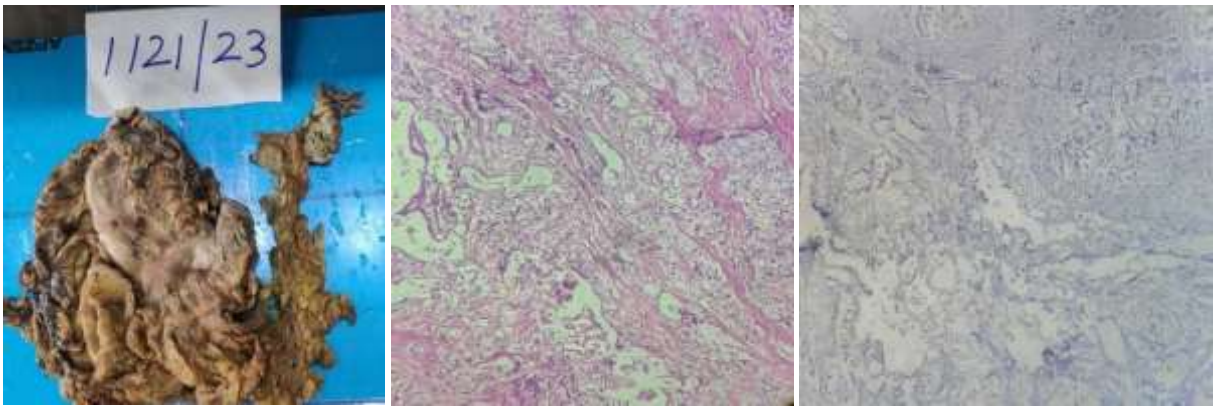

Figure 3: Mucinous carcinoma. (a) Macroscopic view of proliferative growth in the rectosigmoid region, (b) Microscopic image (10x magnification, Hematoxylin & Eosin stain) showing tumour cells in small islands and as single cells within mucin pools, and (c) Immunohistochemical stain for SATB2 (10x magnification) showing no nuclear staining (score 0).

Table 4: Association of SATB2 expression with nodal metastasis (N=37)

Lymph Node Metastasis	SATB2 Positive (n=23)	SATB2 Negative (n=14)	Total (n=37)	P value
Yes	7 (28%)	11(78.5%)	18(48.6%)	0.004*
No	16(62%)	3(21.5%)	19(51.4%)	

According to the available data regarding lymph node metastasis for 37 specimens, it was observed that the specimens exhibiting lymph node metastasis show diminished SATB2 expression levels in comparison to those specimens devoid of lymph node metastasis, which is statistically significant as shown in table 4.

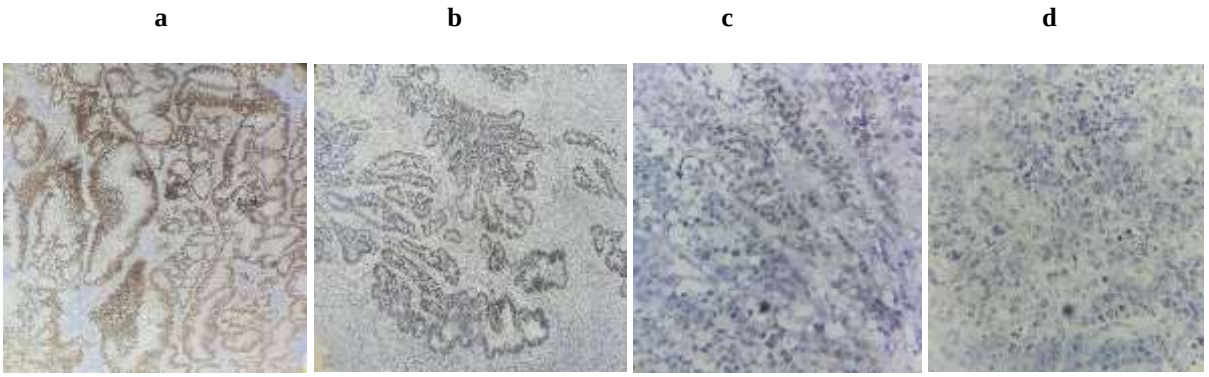

Figure 4: SATB2 expression (a) SATB2 score 6 {strong staining in >75% of cells, (3+3=6)}, (b) SATB2 score 4 {weak staining in >75% of cells, (1+3=4)}, (c) SATB2 score 2 {weak staining in <25% of cells, (1+1=2)}, (d) SATB2 score 0 (absent nuclear staining in all the cells); (Images from left to right).

4. DISCUSSION

A significant number of colorectal carcinoma cases have been distinctly characterized by a notably delayed onset of clinical manifestations, coupled with a wide range of progression rates that ultimately result in an increased number of cases being identified at a more advanced stage of development, which in turn complicates treatment options and patient outcomes. A multitude of comprehensive studies have rigorously established the critical importance of utilizing newly identified molecular markers, which serve not only to evaluate the prognosis of the disease with a higher degree of accuracy but also to facilitate more effective and tailored management strategies for patients suffering from this condition. The ongoing identification and characterization of novel molecules and proteins that play a crucial role in the underlying pathophysiology, as well as the stepwise progression of colorectal carcinomas are instrumental in assessing the prognostic relevance of these factors and in pinpointing innovative therapeutic targets that may significantly enhance treatment efficacy for this prevalent disease.

In the current study, out of the total collected specimens 87% of the study specimens belonged to the age more than 50 years and more than half of the specimens of colorectal carcinomas were male gender of origin. These findings are consistent with the observations of similar clinic pathological observational studies conducted by Wang S et al.[12] With regards to association of SATB2 expression with clinic-pathological parameters, it was found that significant decreased expression of SATB2 was found in the specimens of male gender. M Suvaitha et al., et al also showed the similar pattern of gender association with SATB2 expression.[13] In the present study there is significant association of SATB2 expression was observed with age of the patient more than 50 years and side of obtained specimen was left side. In addition, there is a striking difference in SATB2 expression across different tumor grades. Wel differentiated(G1) tumors predominantly show SATB2 positivity, while poorly differentiated(G3) tumors are more likely to be SATB2 negative (though the sample size for G3 is small) suggesting that SATB2 expression is related to tumor differentiation and aggressiveness. This significant decreased expression of SAT2 with tumor grading was similarly identified by a M Suvaitha et al.[13]

In the 40 resected colorectal adenocarcinoma specimens, decreased SATB2 expression was significantly associated with lymphovascular invasion, a finding consistent with prior research by Li et al., Eldeeb et al., and Ma C et al.[16,17,18] Given the established link between advanced stage, lymphovascular invasion, and poor prognosis, SATB2 expression may serve as an independent prognostic indicator. While previous studies by Yang et al. and Liebig et al[19,20] have demonstrated perineural invasion as an independent prognostic factor, this study did not find a significant association between perineural invasion and SATB2 expression, aligning with the results reported by Li et al. and Eldeeb et al.[16,17] Our study observed that, among the 37 specimens with available lymph node data, 18 cases shown lymph node metastasis, among which 11 cases shown decreased SATB2 expression. This significant decreased expression of SAT2 with lymph node metastasis was similarly identified by a M Suvaitha et al.[13]

5. CONCLUSION

In the current study, it was found that decreased SATB2 expression correlates with regional lymph node metastasis, lymphovascular invasion, and higher tumor grade at diagnosis, all of which serve as indicators of unfavorable prognosis. Therefore, the current research suggests that the reduced expression of SATB2 in tumour cells are linked to a poor prognosis in cases of colorectal adenocarcinoma. This finding can facilitate patient stratification and support personalized treatment strategies aimed at achieving optimal outcomes and enhancing the quality of life for patients.

Conflict of interest

No potential conflicts of interest.

Funding statement

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