Satb2 Expression In Colorectal Carcinoma And Its Association With Grade And Stage Of Tumor

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ABSTRACT

Background: Colorectal carcinoma (CRC) is one of the most prevalent malignancies worldwide and a leading cause of cancer-related mortality. Special AT-rich sequence-binding protein 2 (SATB2) has emerged as a promising biomarker for colorectal epithelial differentiation and prognosis. This study aimed to evaluate the immunohistochemical expression of SATB2 and its correlation with tumor grade and stage of CRC.(1,2)

Methods: A cross-sectional study was conducted on 60 cases of histologically confirmed colorectal carcinoma. Formalin-fixed paraffin-embedded (FFPE) tissue sections were stained with SATB2 antibody, and expression was scored based on nuclear staining extent and intensity. Statistical analysis was performed using Chi-square and Fisher's exact tests, with $p < 0.05$ considered significant.

Results: SATB2 expression was significantly associated with histological grade and tumor stage. Well-differentiated adenocarcinomas showed strong nuclear positivity, whereas poorly differentiated tumors demonstrated loss of SATB2 expression. Moderately differentiated and mucinous adenocarcinomas showed variable staining. SATB2 expression declined with increasing tumor stage, with higher positivity observed in early stages compared to advanced disease.

Conclusion: SATB2 is a reliable immunohistochemical marker in CRC and shows significant correlation with tumor grade and stage. It may serve as a useful diagnostic and prognostic biomarker, aiding in early detection and guiding therapeutic strategies.

KEYWORDS: *Immunohistochmical staining, SCORING IMMUNOREACTIVITY.*

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1. INTRODUCTION

Colorectal carcinoma (CRC) is the third most common cancer globally and the fourth leading cause of cancer-related deaths.(1,2) Its high incidence and mortality emphasize the urgent need for early diagnostic and prognostic markers. Despite advances in treatment, late-stage diagnosis and tumor aggressiveness remain major challenges. SATB2, a nuclear matrix-associated transcription factor, plays an important role in chromatin remodeling and gene expression regulation. Its expression is largely restricted to colorectal epithelium, making it a potential diagnostic marker. Loss of SATB2 has been linked to poor differentiation, tumor aggressiveness, and unfavorable prognosis. This study evaluates SATB2 immunohistochemical expression in CRC and its association with grade and stage.(3,4)

2. MATERIALS AND METHODS

The study was carried out at Sree Balaji Medical College and Hospital (SBMCH), Chromepet, in the Department of Pathology from August 2023 to April 2025. Before beginning the study, approval was taken by the Institutional Human Ethical Committee, SBMCH. Included were 60 Formalin-fixed paraffin-embedded (FFPE) tissue slices from surgically resected specimens and colonoscopic resected and biopsies that were received in our department throughout the study period and sent for histopathology.

Immunohistochmical staining:
It involves two steps. The primary antibody attaches to the particular antigen in the tissue under examination in the first step. Using a secondary antibody and a colorimetric reaction, the primary antibody bound to the antigen is recognised in the second step. The characteristic hue is caused by the reaction of horseradish peroxidase with the secondary enzyme antibody and a dextran polymer. The antigen is displayed in tissue slices, and through particular immunological interactions, the antigen is linked to a visible marker. The process of demonstrating antigen in tissue sections through immunological interactions results in the antigen reacting with DAB (3-3 Diaminobenzene) and attaching a visible marker to it. The conjugation of substrate chromogen produces the hue brown. The protocol for immunohistochemistry was followed.

SCORING IMMUNOREACTIVITY:
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

3. RESULTS AND OBSERVATIONS

The study population comprised 60 patients diagnosed with colorectal carcinoma. The mean age was 46.4 ± 9.2 years, with a higher prevalence in individuals above 60 years (56.7%). A male predominance was observed (63.3%), with a male-to-female ratio of 1.7:1. The sigmoid colon was the most common site of involvement, followed by ascending and transverse colon.

Histologically, adenocarcinoma not otherwise specified was the predominant subtype (70%), followed by mucinous adenocarcinoma (18.3%) and signet ring cell carcinoma (11.7%). With respect to tumor grade, 26.7% were well-differentiated, 46.6% were moderately differentiated, and 26.7% were poorly differentiated. Most tumors were diagnosed in stage II (35%) and stage III (40%), with a smaller proportion in stage I (15%) and stage IV (10%).

Immunohistochemical analysis of SATB2 expression revealed distinct patterns across tumor grades. Well-differentiated adenocarcinomas demonstrated strong and diffuse nuclear positivity (>75% cells; intensity 3+). Moderately differentiated adenocarcinomas showed moderate nuclear staining (26–75% cells; intensity 2+). Poorly differentiated carcinomas largely lacked SATB2 expression (0–1% cells; intensity 0), with only focal weak positivity in some cases. Mucinous and signet ring cell carcinomas showed weak nuclear staining (2–25% cells; intensity 1+).

Correlation with tumor stage showed that early-stage CRCs (stage I and II) retained strong SATB2 positivity, while advanced-stage tumors (stage III and IV) exhibited marked reduction or loss of SATB2 expression. Lymphovascular invasion and nodal metastasis were also associated with reduced SATB2 expression. Chi-square and Fisher's exact tests demonstrated statistically significant associations between SATB2 expression (extent and intensity) and both histological grade ($p < 0.01$) and stage ($p < 0.05$).
Age- and sex-related analysis showed no significant correlation with SATB2 expression, suggesting that its expression is primarily influenced by tumor biology rather than demographic factors. Similarly, tumor site (right vs. left colon) did not significantly impact expression.

Representative micrographs illustrated that well-differentiated adenocarcinomas showed uniform nuclear staining, whereas poorly differentiated carcinomas and mucinous/signet ring variants lacked consistent expression. The declining pattern of SATB2 staining with increasing grade and stage suggests its potential utility as a prognostic marker. Overall, the results strongly support the role of SATB2 as a diagnostic and prognostic biomarker in colorectal carcinoma.

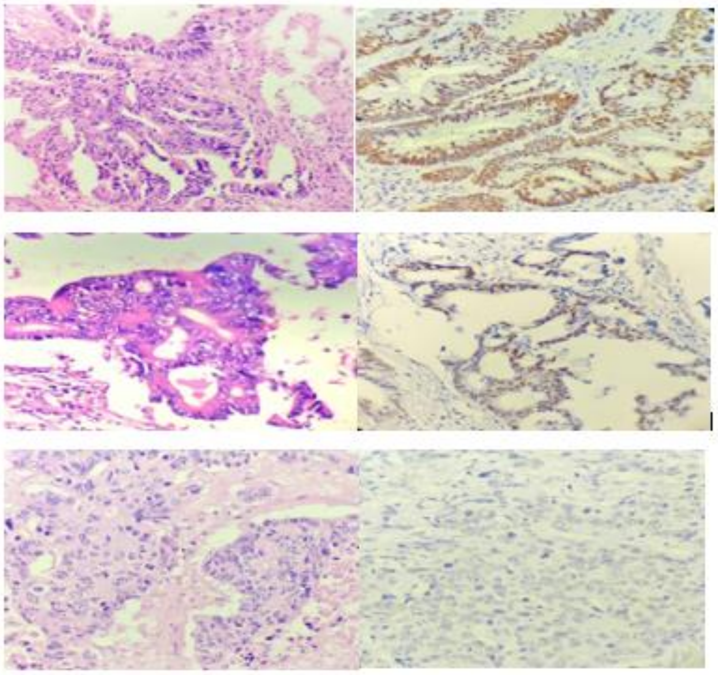


Figure 1&2: well differentiated Adenocarcinoma with intensity of nuclear staining 3+.

Figure 3&4-moderately differentiated Adenocarcinoma with intensity of nuclear staining 2+.

Figure 5&6- poorly differentiated Adenocarcinoma with intensity of nuclear staining 1+.

4. DISCUSSION

Colorectal carcinoma (CRC) is the third most common malignancy worldwide and the second leading cause of cancer-related deaths, with nearly 1.9 million new cases and over 935,000 deaths in 2020. (1,2). The search for sensitive and specific biomarkers has highlighted SATB2, a nuclear matrix-associated transcription factor. Initially described in osteoblast and craniofacial differentiation (Wang et al., 2009), it was later recognized as a highly tissue-specific marker of colorectal epithelium (Magnusson et al., 2011) (12). Positive staining for SATB2 has been reported in over 95% of CRCs (Lin et al., 2014; Wang et al., 2009), confirming its diagnostic reliability. Eberhard et al. (2012) showed that SATB2 overexpression enhanced benefit from adjuvant therapy, while Dragomir et al. (2013) (7) demonstrated its role as a marker of glandular differentiation. Conversely, reduced SATB2 expression has been linked to poor prognosis (Magnusson et al., 2011; Dragomir et al., 2018).(7,12)

Table 2: extent of nuclear staining and its association with sex.

Sex		Extent of Nuclear Staining				Total
		1+	2+	3+	O	
Female	Frequency	4	3	1	2	10
	% within Sex	40.00%	30.00%	10.00%	20.00%	100.00%
Male	Frequency	5	13	31	1	50
	% within Sex	10.00%	26.00%	62.00%	2.00%	100.00%
Total	Frequency	9	16	32	3	60
	% within Sex	15.00%	26.70%	53.30%	5.00%	100.00%
Fisher's Exact Chi-square Test =14.675, P Value <0.01						

Table 3: extent of nuclear staining and its association with histological subtype.

Histological Subtype		Extent Of Nuclear Staining				Total
		1+	2+	3+	O	
Infiltrating Mod.diff Adeno Ca	Frequency	1	10	5	0	16
	% within Histological Subtype	6.30%	62.50%	31.30%	0.00%	100.00%
Mucinous Adeno Ca	Frequency	3	3	1	0	7
	% within Histological Subtype	42.90%	42.90%	14.30%	0.00%	100.00%

Poorly Diff Adenocarcinoma	Frequency	2	0	0	2	4
	% within Histological Subtype	50.00%	0.00%	0.00%	50.00%	100.00%
Signet Ring Cell Carcinoma	Frequency	1	1	0	0	2
	% within Histological Subtype	50.00%	50.00%	0.00%	0.00%	100.00%
Well. Diff. Adeno. Ca	Frequency	2	2	26	1	31
	% within Histological Subtype	6.50%	6.50%	83.90%	3.20%	100.00%
Total	Frequency	9	16	32	3	60
	% within Histological Subtype	15.00%	26.70%	53.30%	5.00%	100.00%
Fisher's Exact Chi-square Test =43.214, P Value <0.01						

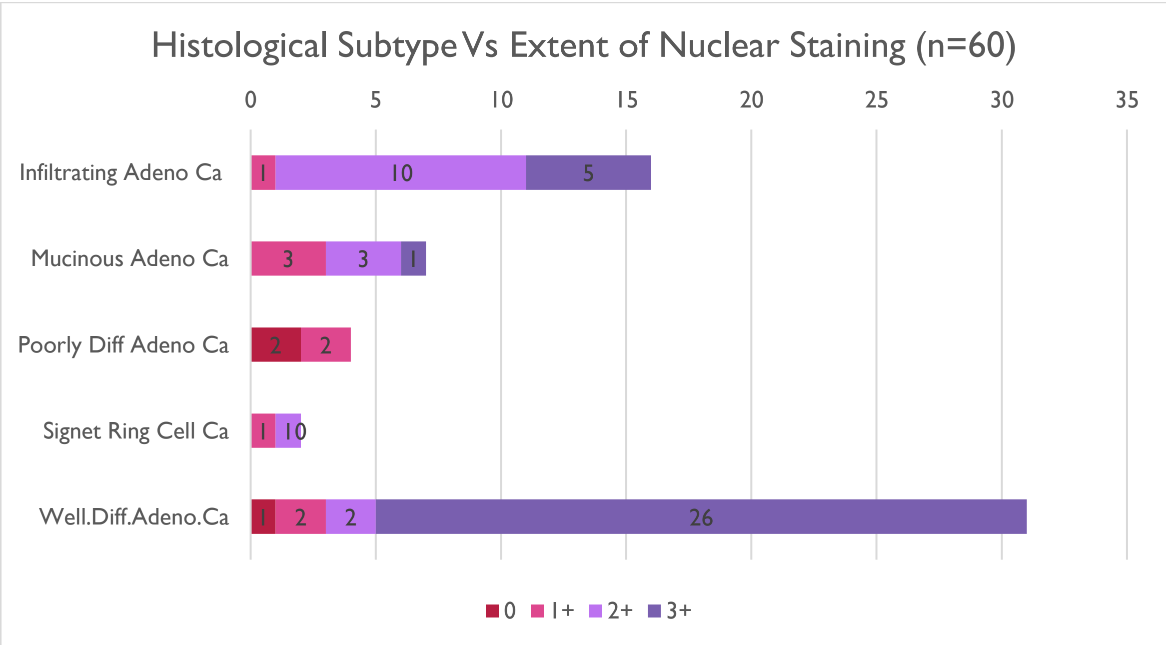


Table 4: extent of nuclear staining and its association with grade.

Grade		Extent Of Nuclear Staining				Total
		1+	2+	3+	O	
Grade 1	Frequency	1	1	13	1	16
	% within Grade	6.30%	6.30%	81.30%	6.30%	100.00%
Grade 2	Frequency	2	6	1	0	9
	% within Grade	22.20%	66.70%	11.10%	0.00%	100.00%
Grade 3	Frequency	4	3	0	1	8
	% within Grade	50.00%	37.50%	0.00%	12.50%	100.00%
Total	Frequency	7	10	14	2	33
	% within Grade	21.20%	30.30%	42.40%	6.10%	100.00%
Fisher's Exact Chi-square Test =23.64, P Value <0.01						

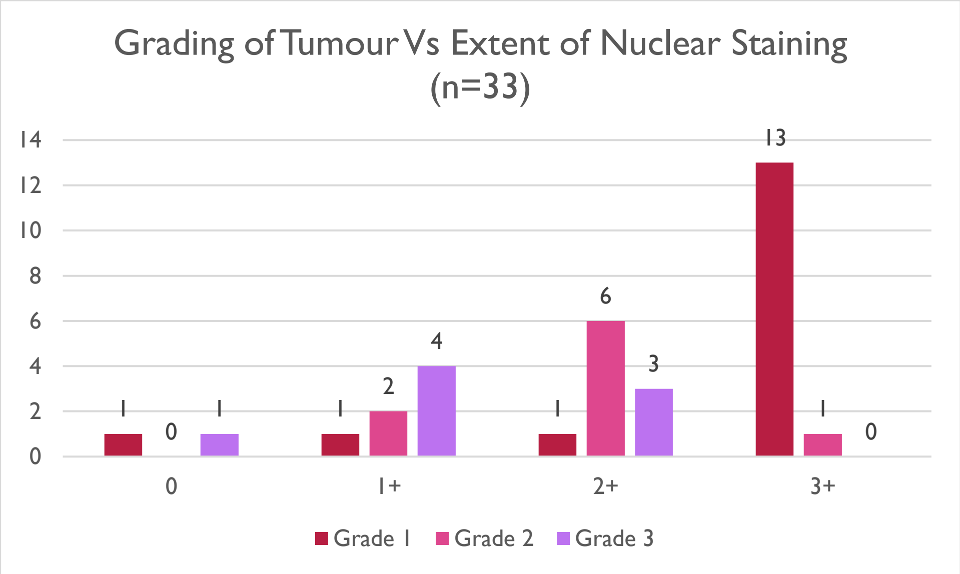


Table 4: Intensity of nuclear staining and its association with sex.

Sex		Intensity of Nuclear Staining				Total
		1+	2+	3+	O	
Female	Frequency	3	4	0	3	10
	% within Sex	30.0%	40.0%	0.0%	30.0%	100.0%
Male	Frequency	10	13	26	1	50
	% within Sex	20.0%	26.0%	52.0%	2.0%	100.0%
Total	Frequency	13	17	26	4	60
	% within Sex	21.7%	28.3%	43.3%	6.7%	100.0%
Fisher's Exact Chi-square Test =14.91, P Value =0.01						

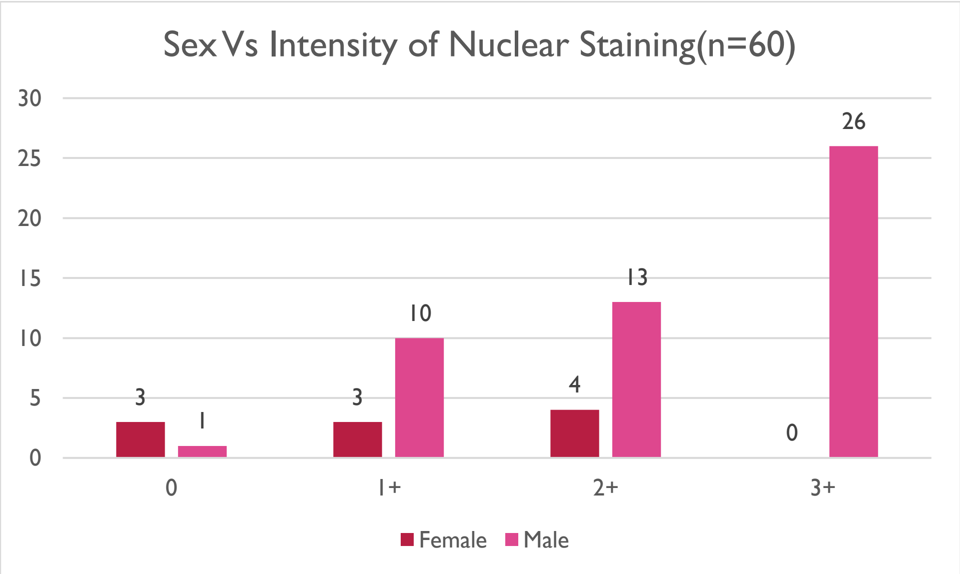


Table 5: Intensity of nuclear staining and its association with histological subtype.

Histological Subtype		Intensity of Nuclear Staining				Total
		1+	2+	3+	O	
Infiltrating mod. diff. Adeno Ca	Frequency	2	11	3	0	16
	% within Histological Subtype	12.5%	68.8%	18.8%	0.0%	100.0%
Mucinous Adeno Ca	Frequency	6	0	1	0	7

	% within Histological Subtype	85.7%	0.0%	14.3%	0.0%	100.0%
Poorly Diff Adenocarcinoma	Frequency	1	0	0	3	4
	% within Histological Subtype	25.0%	0.0%	0.0%	75.0%	100.0%
Signet Ring Cell Carcinoma	Frequency	0	2	0	0	2
	% within Histological Subtype	0.0%	100.0%	0.0%	0.0%	100.0%
Well. Diff. Adeno. Ca	Frequency	4	4	22	1	31
	% within Histological Subtype	12.9%	12.9%	71.0%	3.2%	100.0%
Total	Count	13	17	26	4	60
	% within Histological Subtype	21.7%	28.3%	43.3%	6.7%	100.0%
Fisher's Exact Chi-square Test =46.571, P Value <0.01						

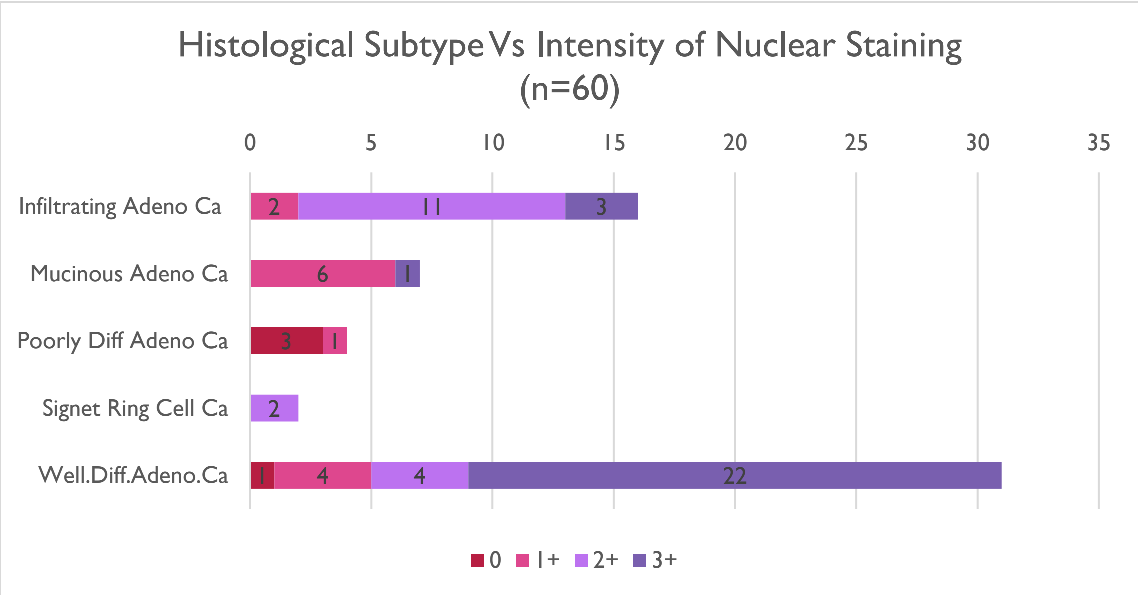
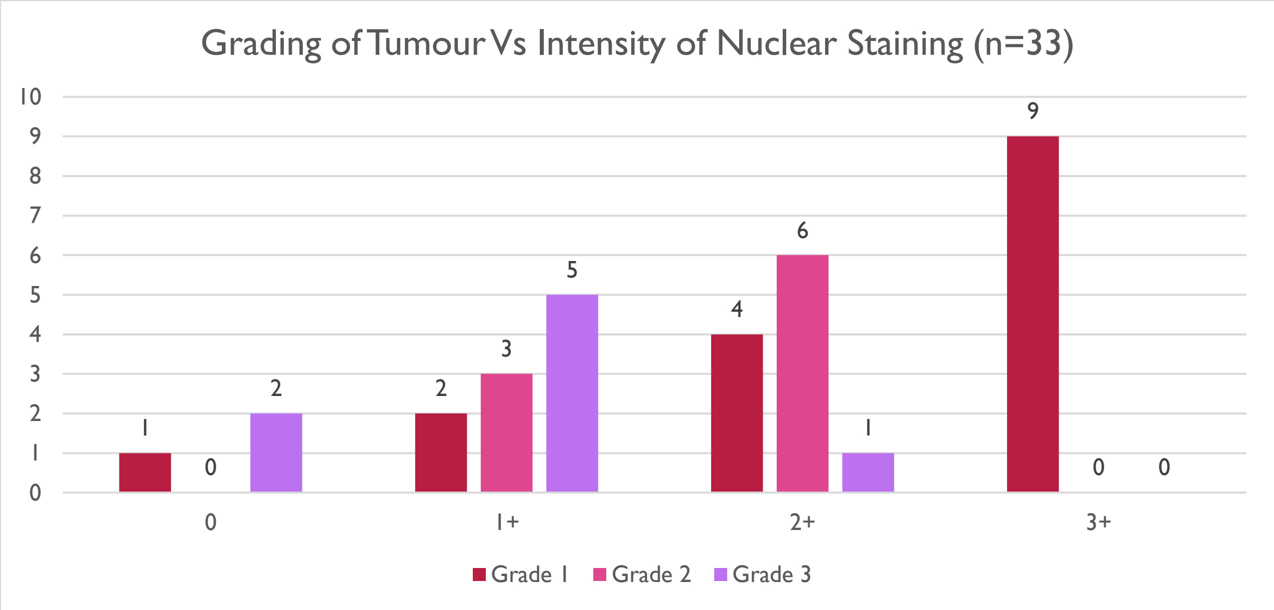


Table 6: Intensity of nuclear staining and its association with grade of the CRC.

Grade		Intensity of Nuclear Staining				Total
		1+	2+	3+	O	
Grade 1	Frequency	2	4	9	1	16
	% within Grade	12.5%	25%	56.3%	6.3%	100.0%
Grade 2	Frequency	3	6	0	0	9
	% within Grade	33.3%	66.7%	0.0%	0.0%	100.0%
Grade 3	Frequency	5	1	0	2	7
	% within Grade	62.5%	12.5%	0.0%	25%	100.0%
Total	Frequency	10	11	9	3	33
	% within Grade	30.3%	33.3%	27.3%	9.1%	100.0%
Fisher's Exact Chi-square Test =18.895, P Value =0.01						



In the present study of 60 CRC cases, SATB2 expression was analyzed across clinicopathological variables. The majority of patients were above 60 years (56.7%), but age was not significantly associated with SATB2 expression, consistent with previous studies (Yang et al., 2018; Ma Henn et al., 2019; Mezheyeuski et al., 2020). Males comprised 83.3% of the study population, and a significant association was found between sex and SATB2 expression ($p<0.01$). This finding contrasts with earlier reports (Suvaitha et al., 2023),(8) which found no sex-based correlation. Laterality analysis showed no association with extent of staining, though intensity was significantly lower in right-sided tumors ($p=0.026$), consistent with observations that tumor sidedness reflects distinct molecular and biological features (Arnold et al., 2017; Lee et al., 2021)(6).

Histological subtype demonstrated the strongest correlation with SATB2 expression. Well-differentiated adenocarcinomas showed strong positivity (3+) in over 80% of cases, confirming SATB2 as a robust marker of glandular differentiation. Moderately differentiated tumors displayed weaker staining, while mucinous adenocarcinomas, poorly differentiated adenocarcinomas, and signet ring cell carcinomas exhibited markedly reduced or absent expression. These findings agree with Dragomir et al. (2013), Miettinen et al. (2014), and Saqi et al. (2021),(10,11,12) who emphasized SATB2's diagnostic utility in conventional adenocarcinomas but noted reduced expression in aggressive or mucinous subtypes. The marked loss of SATB2 in poorly differentiated tumors likely reflects dedifferentiation and loss of glandular phenotype, thereby limiting its diagnostic value in these settings.

Tumor grade also showed a strong and statistically significant association with SATB2 expression. Grade 1 tumors demonstrated the highest frequency of strong positivity, whereas Grade 2 tumors exhibited reduced intensity, and Grade 3 tumors largely lost SATB2 expression. This pattern aligns with reports by Magnusson et al. (2011) and Dragomir et al. (2018),(12) who documented reduced expression in high-grade tumors. Liu et al. (2015) further demonstrated that SATB2 regulates genes involved in differentiation and inhibits tumor progression, supporting the concept that its loss reflects dedifferentiation. Thus, SATB2 serves not only as a diagnostic marker but also as a surrogate marker of tumor differentiation.

Regarding tumor stage, no statistically significant association was found between T stage and SATB2 expression. Nonetheless, early-stage tumors more frequently retained strong staining compared to advanced stages, a trend also observed by Suvaitha et al. (2023).(8) Nodal status, however, showed a significant association ($p=0.017$), with SATB2 expression varying across nodal groups. While some studies suggest loss of SATB2 in advanced tumors (Magnusson et al., 2011; Suvaitha et al., 2023),(8,12) our findings suggest that expression may persist even in node-positive cases. Lymphovascular invasion did not significantly correlate with SATB2 expression, although weaker staining was more common in LVI-positive tumors, echoing Magnusson et al. (2011),(12) who linked reduced SATB2 to adverse pathological features.

Overall, these findings confirm SATB2 as a highly sensitive marker for colorectal adenocarcinoma, especially in well-differentiated tumors. Its strong expression in conventional adenocarcinomas makes it valuable for confirming colorectal origin, particularly in distinguishing from extraintestinal primaries. However, its expression is reduced in mucinous, poorly differentiated, and signet ring cell subtypes, limiting its diagnostic reliability in such cases. The significant associations with histological subtype, grade, sex, laterality, and nodal status suggest that SATB2 reflects both differentiation and aspects of tumor biology. Reduced or absent expression in high-grade tumors underscores its prognostic relevance, indicating more aggressive behavior.

In summary, SATB2 is a valuable biomarker in colorectal carcinoma, strongly expressed in well-differentiated adenocarcinomas and reduced in aggressive variants. Its expression correlates with histological subtype, grade, sex, laterality, and nodal status, but not with age, T stage, or lymphovascular invasion. These results, in line with prior studies (Magnusson et al., 2011; Dragomir et al., 2018; Suvaitha et al., 2023),(12,8) highlight its diagnostic and potential prognostic roles. While SATB2 remains highly reliable for conventional adenocarcinomas, caution is required when interpreting its absence in poorly differentiated and mucinous tumors, where loss of expression may complicate diagnosis and signal aggressive disease biology.

5. CONCLUSION

The present study highlights the diagnostic and prognostic value of SATB2 in colorectal carcinoma. Our findings demonstrate strong SATB2 expression in well-differentiated CRCs and reduced or absent expression in poorly differentiated tumors, consistent with previous studies that identified SATB2 as a lineage-specific marker for colorectal differentiation. SATB2 expression decline with increasing tumor stage further supports its role as a prognostic biomarker. Mucinous and signet ring variants showed weaker expression, reflecting their aggressive biological behaviour. SATB2 expression is significantly correlated with colorectal carcinoma grade and stage. It is strongly expressed in well-differentiated tumors and decreases with higher grade and advanced stage. Hence, SATB2 is a promising biomarker that can aid in the diagnosis, prognostication, and possibly therapeutic stratification of CRC patients.

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