**Original Article**
Middle East Journal of Cancer; January 2024; 15(1): 15-24

# Prognostic Significance of Notch-4, SATB-2, and Glutaminase-1 in Colorectal Adenocarcinoma

**Noha F. Elaidy*, PhD, Amira Elwan**, PhD, Mohamed Wahba Hegazy**♦, PhD, Hanaa A. Atwa*, PhD**

**Pathology Department, Medicine College, Zagazig University, Egypt*
***Department of Clinical Oncology and Nuclear Medicine, Medicine College, Zagazig University, Egypt*

Please cite this article as: Elaidy NF, Elwan A, Hegazy MW, Atwa HA. Prognostic significance of Notch-4, SATB-2, and glutaminase-1 in colorectal adenocarcinoma. Middle East J Cancer. 2024; 15(1):15-24. doi: 10.30476/mejc.2023.97207.1852.

## Abstract

**Background:** Neurogenic locus notch homology 4 (Notch-4) is crucial in maintaining stem cells. Special AT-rich sequence-binding protein 2 (SATB-2) is a transcription factor that binds to the nuclear matrix and serves various functions, including brain development. Glutaminase-1 (GLS-1) plays a pivotal role in cancer cell metabolism, growth, and proliferation. This study aims to assess the expression of these markers in colorectal cancer, establishing correlations with clinicopathological characteristics and patient survival.

**Method:** In this retrospective study, we retrieved and analyzed 68 formalin-fixed, paraffin-embedded blocks of primary colorectal adenocarcinoma, not otherwise specified cases, and adjacent normal mucosa. Notch-4, SATB-2 and GLS-1 expressions were analyzed using immunohistochemistry at the Zagazig School of Medicine, Egypt.

**Results:** High expressions of Notch-4 and GLS-1 and low expression of SATB-2 were observed in colonic adenocarcinoma but not in adjacent non-neoplastic mucosa ($P < 0.001$). High expressions of Notch-4 and GLS-1, along with low expression of SATB-2, were associated with a higher tumor grade, advanced stage ($P < 0.001$), lymphovascular invasion, lymph node metastasis, and poor disease-free survival and overall survival rates ($P < 0.001$).

**Conclusion:** High expression of Notch-4 and GLS-1 is correlated with a poor prognosis in colorectal cancer, while high expression of SATB-2 is associated with a favorable prognosis for colorectal carcinoma. These markers can aid in predicting tumor prognosis and guiding targeted therapy for colorectal carcinoma.

***Keywords:*** Colorectal neoplasms, Immunohistochemistry, Prognosis

♦Corresponding Author:
Mohamed Wahba Hegazy, PhD
Department of Clinical Oncology and Nuclear Medicine, Medicine College, Zagazig University, Egypt
Tel: +201090063872
Email: F1501625@gmail.com

## Introduction

Colorectal cancer (CRC) is a prevalent malignant disease occurring worldwide and ranks the second leading cause of cancer-related deaths.[1] In Egypt, CRC was detected in 11%–15% of patients undergoing colonoscopy.[2] Despite

Received: November 08, 2022; Accepted: February 26, 2023

significant advances in early diagnosis, surgical interventions, and chemotherapy agents, many cases will develop metastases and experience recurrence.[3]

Cancer stem cells are a subset of cells responsible for initiating tumors and can endure radio-chemotherapy, leading to local recurrences and metastasis despite treatment.[4]

Neurogenic locus notch homology 4 (Notch-4) plays a vital role in embryonic development and maintaining stem cells by balancing cell apoptosis and proliferation.[5] Notch-4 can function as both a significant oncogene and a tumor suppressor.[6]

Particular AT-rich sequence binding protein-2 (SATB-2) is a transcription factor that binds to the nuclear matrix, with multiple functions in brain development and osteoblast differentiation.[7]

In epithelial cells, SATB-2 expression is confined to glandular cells in the lower gastrointestinal tract, enabling the identification of over 95% of all CRCs.[8]

High SATB-2 expression correlates with a favorable prognosis in patients with laryngeal, esophageal, pancreatic, and clear-cell renal carcinoma.[9] In CRC, elevated SATB-2 expression is linked to a favorable prognosis and increased sensitivity to chemotherapy and radiation,[10] while lower expressions of SATB-2 are associated with advanced stage, high grade, and tumor recurrence.[11]

Cancer cells display heightened glucose and glutamine metabolism, with glutaminase-1 (GLS-1) converting glutamine to glutamate, playing a pivotal role in cancer cell metabolism, growth, and proliferation.[12] Elevated GLS-1 expression has been observed in various cancer types, including CRC, prostate cancer, and breast cancer.[13, 14] Inhibition of GLS-1 reduces the proliferation rate and suppresses the epithelial-mesenchymal transition process.[15]

This study investigates the correlation between Notch-4, SATB-2, and GLS-1 in CRC and clinicopathological characteristics and their impact on patient survival.

## Materials and Methods

This is a retrospective analysis that includes 68 formalin-fixed, paraffin-embedded tissue blocks of primary CRC, not otherwise specified, according to the WHO classification of 2019.

These cases, along with adjacent normal mucosa, were retrieved from the archives of the Pathology Department at the College of Medicine, Zagazig University, Egypt, spanning from December 2014 to December 2018. Immunohistochemical antibodies to Notch-4, SATB-2, and GLS-1 were utilized in this study. Written informed consents were obtained from all patients, and the research was conducted in compliance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) and received

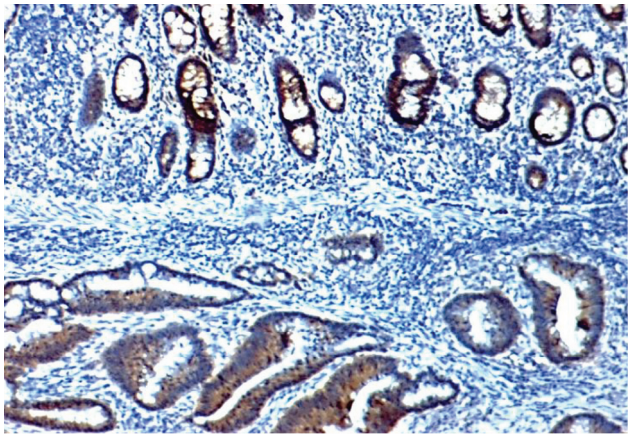

**Figure 1.** Well-differentiated adenocarcinoma showing mild Notch-4 immunoreactivity with negative expression in normal colonic mucosa (ABC, ×100).
Notch-4: Neurogenic locus notch homology 4

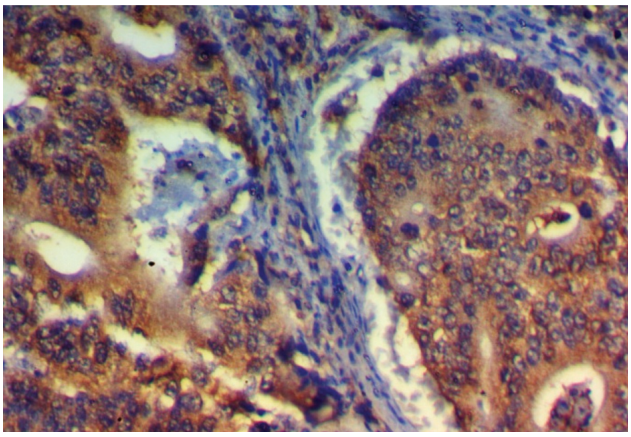

**Figure 2.** Poorly-differentiated adenocarcinoma showing strong Notch-4 immunoreactivity (ABC, ×100).
Notch-4: Neurogenic locus notch homology 4

approval from the Ethical Research Committee of the Faculty of Medicine at Zagazig University (IRB#9805/18-9-2022).

Complete clinical data were acquired from the patient's medical records. The grading of CRC was determined based on the criteria published by WHO in 2010, and staging was performed using the TNM staging system. Various chemotherapy protocols like fluorouracil or capecitabine with oxaliplatin were recommended for 56 patients with high-risk stages II and III. Additionally, 23 patients diagnosed with rectal carcinoma underwent concurrent chemoradiotherapy, chemoradiotherapy by 3-dimensional conformal radiotherapy using 50 Gy in 25 fractions, and follow-up was conducted at the Department of Clinical Oncology and Nuclear Medicine at Zagazig University, Egypt.

### *Immunohistochemical staining*

The Streptavidin-biotin technique was employed, utilizing a primary mouse monoclonal antibody targeting Notch-4 (DakoCytomation, Carpinteria, CA, USA, used at a 1:50 dilution), a rabbit polyclonal primary anti-SATB-2 antibody (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA, used at a dilution of 1:100), and a rabbit polyclonal antibody directed against GLS-1 (ab93434, Abcam, USA, used at a dilution of 1:100).

### *Evaluation of immunoexpression*

Regarding Notch-4 staining, cytoplasmic staining intensity was categorized as 1+, 2+, and 3+. The percentage of positive cells was classified into four categories: 0 for 0%, 1 for 1%-33%, 2 for >33%-66%, and 3 for >66%.16

For SATB-2 expression, nuclear staining was assessed using a 4-tier intensity scale: 1 = negative, 2 = weak, 3 = moderate, or 4 = vigorous. The estimated fraction of stained tumor cells was denoted as 1 (0%–1%), 2 (2%–10%), 3 (11%–25%), 4 (26%–50%), 5 (51%–75%), and 6 (>75%).[17]

GLS-1: Membrane expression was scored as follows: 0 (negative, no positive cells), 1 (minimal, <10% positive cells), 2 (moderate, 10%–40% positive cells), 3 (strong, 40%–70% positive cells), and 4 (maximum, >70% positive cells).[18]

### *Statistical analysis*

Statistical analysis was performed using SPSS version 22.0 for Windows (SPSS Inc., Chicago, IL, USA) and MedCalc Software bvba 13 for Belgium (Ostend). Categorical variables' percentages were compared using Pearson's Chi-square or Fisher's exact tests. The Mann-Whitney U test was employed to compare two groups of non-normally distributed variables. Disease-free survival (DFS) and overall survival (OS) were assessed using the Kaplan-Meier curve and log-rank test. $P < 0.05$ was considered statistically significant.

## Results

Patient's characteristics are shown in table 1, grade I and stage 3 represent 56% and 67.6%,

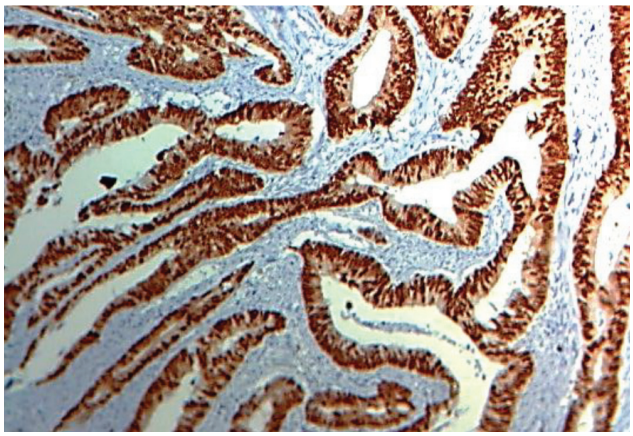

**Figure 3.** Well-differentiated adenocarcinoma showing strong SATB-2 immunoreactivity (ABC, ×100).
SATB-2: Special AT-rich sequence-binding protein 2

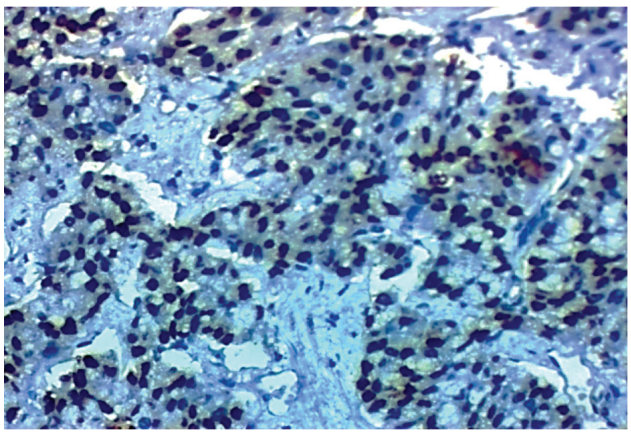

**Figure 4.** Poorly-differentiated adenocarcinoma showing mild SATB-2 immunoreactivity (ABC, ×400).
SATB-2: Special AT-rich sequence-binding protein 2

respectively. Positive lymph nodes were common in 73.5% of cases.

*Immunohistochemical results*

As shown in tables 2 and 3, Notch-4 was stained in the cytoplasm of cancer cells (Figures 1 and 2), STAB-2 exhibited nuclear expression (Figures 3 and 4), and GLS-1 expression was membranous (Figures 5 and 6).

In this study, Notch-4 was negative in normal mucosa but only detected in the blood vessels. Notch-4 was expressed in 45.6% of all cases. In the more differentiated adenocarcinoma, Notch-4 was less expressed than in poorly differentiated adenocarcinoma (47.4% versus 77.8%).

There is a statistically significant relationship between positive Notch-4 expression and both lymphovascular invasion (LVI) and lymph node involvement ($P = 0.041$ and $< 0.001$, respectively). Also, a statistically significant relationship exists between positive Notch-4 expression and tumor stage ($P = 0.001$).

SATB-2 positive expression was statistically correlated with negative lymph node involvement, low grade, and tumor stage ($P = 0.001$, $<0.001$, and 0.04, respectively).

GLS-1 membranous expression was detected in 57.4% of cases. In the more differentiated adenocarcinoma, GLS-1 was less expressed than in poorly differentiated adenocarcinoma.

*Recurrence and survival analysis*

Patients who had high Notch-4 and GLS-1 expressions were associated with unfavorable 5-year OS (mean = 32 months ± 16.9, $P = 0.001$) and (mean = 32.3 months ± 16.2, $P < 0.001$), respectively. Unfavorable 5-year DFS (mean = 20.9 months ± 12.9, $P < 0.001$) and (mean = 21.3 months ± 11.5, $P < 0.001$), respectively, while favorable 5-year OS (mean = 49.1 months ± 13.4, $P < 0.001$) and favorable 5-year DFS (mean = 37.4 months ± 14.6, $P < 0.001$) were observed in patients with high SATB-2 expression (Table 3, Figures 7-12).

## Discussion

68 patients were included in this study, with 56% and 67.6% having grade I and stage 3, respectively. Positive lymph nodes were observed in 73.5% of cases. High expressions of Notch-4, GLS-1, and low expression of SATB-2 were found in colonic adenocarcinoma but not in adjacent non-neoplastic mucosa ($P < 0.001$). Notch-4 and GLS-1 high expressions, along with SATB-2 low expression, were associated with high tumor grade, advanced stage ($P < 0.001$), LVI, lymph node metastasis, poor DFS, and OS rates.

Notch families are pivotal in regulating various cellular processes and may serve as biomarkers for cancer diagnosis and prognosis. Notch signaling is essential for forming and self-renewal tumor-initiating cells in colon cancer.[19]

In this study, Notch-4 was negative in normal mucosa but positive in cancer cells, expressed in 45.6% of all cases. Well-differentiated adenocarcinoma exhibited lower Notch-4 expression than poorly-differentiated adenocarcinoma (47.4% versus 77.8%). Higher

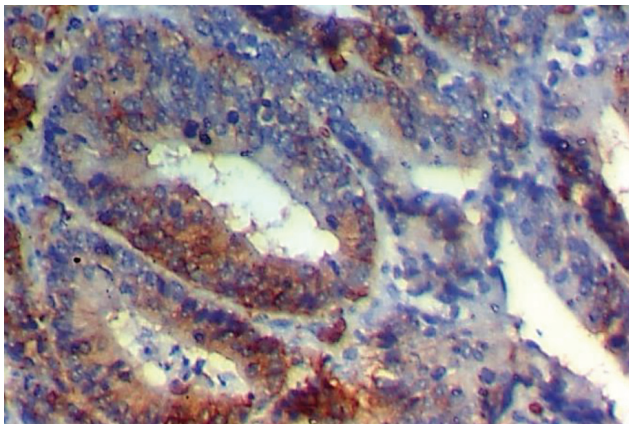

**Figure 5.** Well-differentiated adenocarcinoma showing mild Glutaminase-1 immunoreactivity (ABC, ×100).

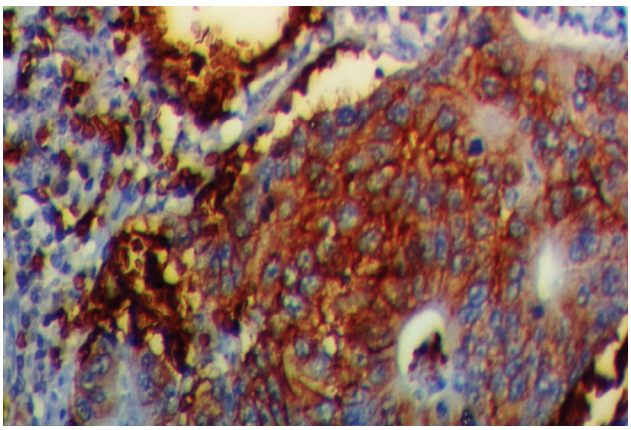

**Figure 6.** Poorly-differentiated adenocarcinoma showing strong Glutaminase-1 immunoreactivity (ABC, ×100).

Notch-4 expression was associated with advanced grade, stage, distant metastases, the presence of LVI, a shorter OS, and higher relapse rate (mean = 32.0 months ± 16.85, $P = 0.001$). Similar findings were reported by Wu et al.[20] Survival analysis indicated that Notch-4 status is an independent prognostic factor for OS and recurrence-free survival in several malignancies beyond colon cancer. Notch-4 can induce epithelial-mesenchymal transition by upregulating HEY1 in head and neck squamous cell carcinoma.[21]

SATB-2 expression acts as a tumor suppressor gene in various tumor types. Mansour et al. revealed that SATB-2 functions as a tumor suppressor gene in CRC by inhibiting ERK5 activation. Low or lack of SATB-2 expression is indicative of malignant behavior and a poor prognosis in CRC.[22]

In this study, SATB-2 was expressed in 86.8% of all cases, aligning closely with Magnusson et al.'s[9] results of SATB-2 expression in 85.8% of primary CRC cases. Another study by Dragomir et al. found that SATB-2 had 93% sensitivity and 77% specificity in supporting colorectal origin,[23] which aligns with the results of Zhang et al., who documented SATB-2 expression in 93% of their studied cases.[24]

In this work, SATB-2 immunohistochemical expression was associated with a favorable prognosis, including a 5-year OS of patients (mean = 49.1 months ± 13.4, $P < 0.001$) and a favorable 5-year DFS (mean = 37.4 months ± 14.6, $P < 0.001$), consistent with the findings of Mezheyeuski et al.[25]

SATB-2 influences tumor growth and spreading through SATB-2-mediated chromatin rearrangement and the differential expression of microRNAs, which affect the expression of genes crucial for migration and invasion.[26] A metastasis-suppressor microRNA, miRNA-31, hinders translation into the SATB-2 protein, correlating with a poorer prognosis in CRC patients.[27]

Cancer cells rely on glutamine for growth, with GLS-1 critical in catalyzing glutaminases. GLS-1 is pivotal in cancer cell metabolism, growth, and proliferation.[12] In the current study,

**Table 1.** Clinicopathological features of the studied patients

| | N=68 | % |
|---|---|---|
| **Age** | | |
| ≤50 years | 19 | 27.9 |
| >50 years | 49 | 72.1 |
| **Gender** | | |
| Male | 36 | 52.9 |
| Female | 32 | 47.1 |
| **Tumor site** | | |
| Right colon | 15 | 22.1 |
| Transverse colon | 14 | 20.6 |
| Left colon | 16 | 23.5 |
| Rectum | 23 | 33.8 |
| **Gross pattern** | | |
| Ulcerating | 22 | 32.4 |
| Fungating | 16 | 23.5 |
| Annular | 30 | 44.1 |
| **Size group** | | |
| <5 cm | 15 | 22.1 |
| 5-10 cm | 12 | 17.6 |
| >10 cm | 41 | 60.3 |
| **Grade** | | |
| 1 | 38 | 55.9 |
| 2 | 21 | 30.9 |
| 3 | 9 | 13.2 |
| **LVI** | | |
| Absent | 61 | 89.7 |
| Present | 7 | 10.3 |
| **Lymph node** | | |
| Negative | 18 | 26.5 |
| Positive | 50 | 73.5 |
| **T** | | |
| T1 | 3 | 4.4 |
| T2 | 18 | 26.5 |
| T3 | 47 | 69.1 |
| **N** | | |
| N0 | 18 | 26.5 |
| N1 | 50 | 73.5 |
| **Stage** | | |
| I | 4 | 5.9 |
| II | 18 | 26.5 |
| III | 46 | 67.6 |
| **Notch-4** | | |
| Negative | 37 | 54.4 |
| Positive | 31 | 45.6 |
| **SATB-2** | | |
| Positive | 59 | 86.8 |
| Negative | 9 | 13.2 |
| **Glutaminase-1** | | |
| Negative | 29 | 42.6 |
| Positive | 39 | 57.4 |
| **Death** | | |
| No | 33 | 48.5 |
| Yes | 35 | 51.5 |
| **Relapse** | | |
| No | 31 | 45.6 |
| Yes | 37 | 54.4 |
| **OS (months)** | | |
| Mean ± SD | 39.8 ± 17.9 | |
| Range | 8 – 60 | |
| **DFS (months)** | | |
| Mean ± SD | 29.37±17.13 | |
| Range | 14- 60 | |

T: Tumor stage; N: Nodal metastasis; LVI: Lymphovascular invasion; OS: Overall survival; DFS: Disease-free survival; Notch-4: Neurogenic locus notch homology 4; SATB-2: Special AT-rich sequence-binding protein 2

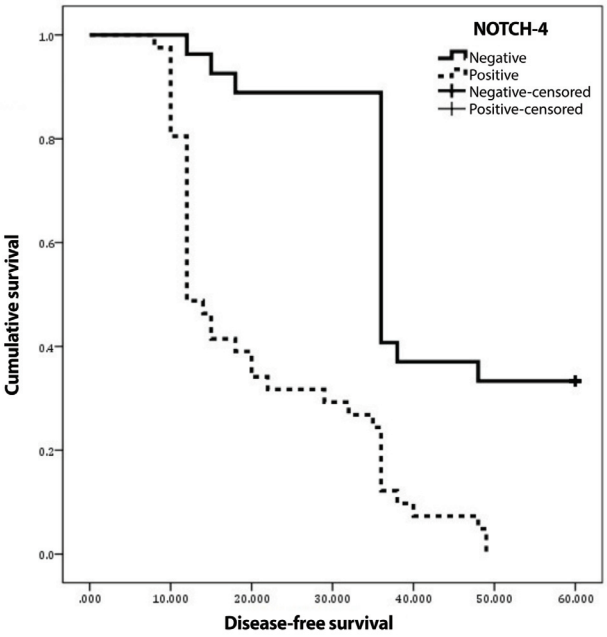


**Figure 7.** Kaplan-Meier plot shows a significant relationship between Notch-4 and disease-free survival.
Notch-4: Neurogenic locus notch homology 4

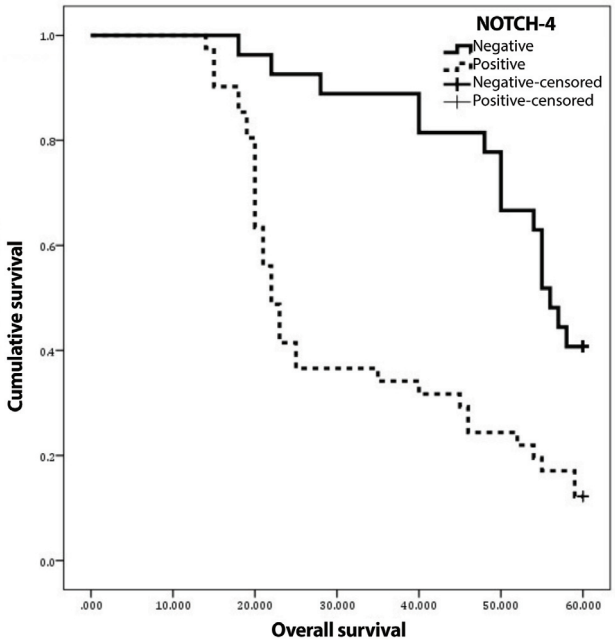


**Figure 8.** Kaplan-Meier plot shows a significant relationship between the Notch-4 level and overall survival.
Notch-4: Neurogenic locus notch homology 4

positive GLS-1 expression was observed in 57.4% of cases, and there was a positive correlation between GLS-1 protein expression and poor clinicopathological features of CRC, including advanced stage and tumor grade.

These results align with the findings of Xiang et al.,[28] who reported that high GLS-1 expression significantly correlated with lymph node metastasis but not with the patient's age, gender, or tumor grade. Huang et al.[29] also demonstrated that GLS-1 expression was associated with distant metastasis, TNM staging, and the histological

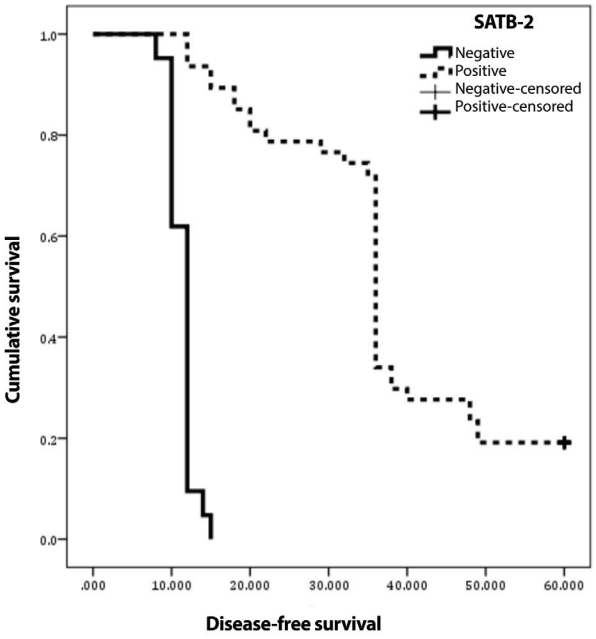


**Figure 9.** Kaplan-Meier plot showing a significant relationship between SATB-2 and disease-free survival.
SATB-2: Special AT-rich sequence binding protein-2

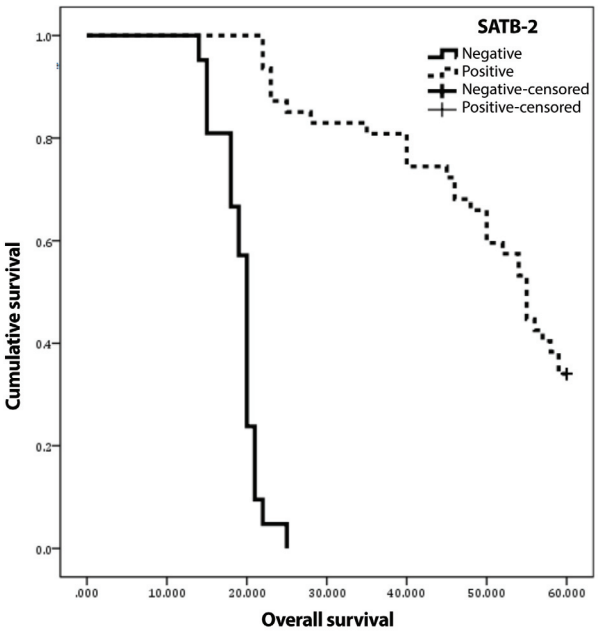


**Figure 10.** Kaplan-Meier plot showing the relationship between SATB-2 and overall survival.
SATB-2: Special AT-rich sequence binding protein-2; Notch-4: Neurogenic locus notch homology 4

**Table 2.** Relation between Notch-4, SATB-2, and Glutaminase-1 expressions in patients and clinicopathological parameters

| | No. | Notch-4 | | | SATB-2 | | | Glutaminase -1 | | P |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Positive N = 37(%) | Negative N = 31(%) | P# | Positive N = 59 (%) | Negative N = 9(%) | P# | Positive N = 29(%) | Negative N = 39(%) | P# |
| **Age group** | | | | | | | | | | |
| ≤50 | 19 | 13 (68.4) | 6 (31.6+) | 0.149 | 16 (84.2) | 3 (15.8) | 0.702 | 7 (36.8) | 12 (63.2) | 0.547 |
| >50 | 49 | 24 (49) | 25 (51) | | 43 (87.8) | 6 (12.2) | | 22 (44.9) | 27 (55.1) | |
| **Sex** | | | | | | | | | | |
| Male | 36 | 17 (47.2) | 4 (52.8) | 0.207 | 31 (86.1) | 5 (13.9) | > 0.099 | 20 (55.6) | 16 (44.4) | 0.022 |
| Female | 32 | 20 (62.5) | 37 (37.5) | | 28 (87.5) | 4 (12.5) | | 9 (28.1) | 23 (71.9) | |
| **Site** | | | | | | | | | | |
| Rt colon | 15 | 10 (66.7) | 5 (33.3) | 0.696 | 12 (80) | 3 (20) | 0.794 | 4 (26.7) | 11(73.3) | 0.086 |
| Transverse | 14 | 7 (50) | 7 (50) | | 12 (85.7) | 2 (14.3) | | 10 (71.4) | 4 (28.6) | |
| LT colon | 16 | 9 (56.2) | 7 (43.8) | | 14 (87.5) | 2 (12.5) | | 6 (37.5) | 10 (62.5) | |
| Rectum | 23 | 11 (47.8) | 12 (52.2) | | 21 (91.3) | 2 (8.7) | | 9 (39.1) | 14 (60.9) | |
| **Gross pattern** | | | | | | | | | | |
| Ulcerating | 22 | 12 (54.5) | 10 (45.5) | 0.911 | 22 (100) | 0 (0) | 0.003* | 5 (22.7) | 17 (77.3) | 0.066 |
| Fungating | 16 | 8 (50) | 8 (50) | | 10 (62.5) | 6 (37.5) | | 9 (56.2) | 7 (43.8) | |
| Annular | 30 | 17 (56.7) | 3(43.3) | | 27 (90) | 3 (10) | | 15 (50) | 15 (50) | |
| **Size** | | | | | | | | | | |
| <5 cm | 15 | 6 (40) | 9 (60) | 0.402 | 14 (93.3) | 1 (6.7) | 0.292 | 7 (46.7) | 8 (53.3) | 0.808 |
| 5-10cm | 12 | 8 (66.7) | 4 (33.3) | | 8 (66.7) | 4 (33.3) | | 5 (41.7) | 7 (58.3) | |
| >10cm | 41 | 23 (56.1) | 18(43.9) | | 37 (90.2) | 4 (9.8) | | 17 (41.5) | 23(58.5) | |
| **Grading** | | | | | | | | | | |
| 1 | 38 | 20 (52.6) | 18 (47.4) | 0.452 | 38 (100) | 0 (0) | <0.001 | 15 (39.5) | 23 (60.5) | 0.419 |
| 2 | 21 | 15 (71.4) | 6 (28.6) | | 19 (90.5) | 2 (9.5) | | 9 (42.9) | 12 (57.1) | |
| 3 | 9 | 2 (22.2) | 7 (77.8) | | 2 (11.1) | 7 (88.9) | | 5 (55.6) | 4 (44.4) | |
| **LVI** | | | | | | | | | | |
| Absent | 61 | 36 (59) | 25 (41) | 0.041 | 59 (96.7) | 2 (3.3) | <0.001 | 25 (41) | 36 (59) | 0.449 |
| Present | 7 | 1 (14.3) | 6 (85.7) | | 0 (0) | 7 (100) | | 4 (57.1) | 3 (42.9) | |
| **LN** | | | | | | | | | | |
| Negative | 18 | 18 (100) | 0 (0) | <0.001 | 11 (61.1) | 7 (38.9) | 0.001 | 14 (77.8) | 4 (22.2) | <0.001 |
| Positive | 50 | 19 (38) | 31 (62) | | 48 (96) | 2 (4) | | 15 (30) | 35 (70) | |
| **T** | | | | | | | | | | |
| T1 | 3 | 3 (100) | 0 (0) | 0.003 | 0 (0) | 3 (100) | <0.001 | 1 (33.3) | 2 (66.7) | 0.039 |
| T2 | 18 | 14 (77.8) | 4 (22.2) | | 13 (72.2) | 5 (22.2) | | 13 (72.2) | 5 (27.8) | |
| T3 | 47 | 20 (42.6) | 27(57.4) | | 46 (97.9) | 1 (2.1) | | 15 (31.9) | 32(68.1) | |
| **N** | | | | | | | | | | |
| N0 | 18 | 17 (94.4) | 1 (5.6) | <0.001 | 11 (61.1) | 7 (38.9) | 0.001 | 13 (72.2) | 5 (27.8) | 0.003 |
| N1 | 50 | 20 (40) | 30 (60) | | 48 (96) | 2 (4) | | 16 (32) | 34 (68) | |
| **Stage** | | | | | | | | | | |
| I | 4 | 4 (100) | 0 (0) | 0.001 | 4 (100) | 0 (0) | 0.04* | 2 (50) | 2 (50) | 0.016 |
| II | 18 | 14 (77.8) | 4 (22.2) | | 18 (100) | 0 (0) | | 13 (72.2) | 5 (27.8) | |
| III | 46 | 19 (41.3) | 27(58.7) | | 37 (80.4) | 9 (19.6) | | 14 (30.4) | 32(69.6) | |

*$P$ <0.05 is statistically significant; #Chi square test; **$P$ ≤0.001 is statistically highly significant; Notch-4: Neurogenic locus notch homology 4; SATB-2: Special AT rich sequence binding protein-2; RT: Right; LT: Left; LN: Lymph node metastasis; LVI: Lymphovascular invasion; T: Tumor stage; N: Nodal; No.: Number

type of CRC.

In this work, high GLS-1 expression was linked to a poorer prognosis, including an unfavorable 5-year OS of patients (mean = 21.3 months ± 11.5, $P < 0.001$), similar to the study by Song et al.[30]

In Egypt, CRC is a significant health concern with a rising number of cases, particularly in young individuals. It was identified in 11%–15% of patients who underwent colonoscopy, and a substantial proportion of cases developed metastases and recurrence. Therefore, novel markers such as Notch-4, SATB-2, and GLS-1 may aid in predicting the prognosis of this type of CRC, potentially reducing morbidity and mortality.

The limitations of this study include its retrospective nature, a small number of cases, and the lack of genetic expression analysis of these markers in CRC.

## Conclusion

High expression of Notch-4 and GLS-1 is correlated with a poor prognosis, while a high expression of SATB-2 is correlated with a good prognosis in CRC. These markers are considered novel targets in the therapy of CRC.

**Table 3.** Relation between Notch-4, SATB-2, and Glutaminase -1expressions in the studied patients and patients' survival

| | Total | Notch-4 | | | SATB-2 | | | Glutaminase -1 | | P |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Positive N = 37(%) | Negative N = 31(%) | P# | Positive N = 59 (%) | Negative N = 9(%) | P# | Positive N = 29(%) | Negative N = 39(%) | P# |
| **Relapse** | | | | | | | | | | |
| Absent | 31 | 26 (83.9) | 5 (16.1) | <0.001 | 30 (77.4) | 1 (22.6) | 0.009 | 19 (61.3) | 12 (38.7) | 0.004 |
| Present | 37 | 11 (29.7) | 26 (70.3) | | 29 (94.6) | 8 (5.4) | | 10 (27) | 27 (73) | |
| **Death** | | | | | | | | | | |
| No | 33 | 26 (78.8) | 7 (21.2) | <0.001 | 25 (75.8) | 8(24.2) | 0.012 | 18 (54.5) | 15 (45.5) | 0.054 |
| Yes | 35 | 11 (31.4) | 24 (68.6) | | 34 (97.1) | 1 (3.9) | | 11 (31.4) | 24 (68.6) | |
| **OS** | | | | | | | | | | |
| Mean ± SD | 39.8 ± 17.9 | 47.7 ± 12.9 | 32.0 ± 16.9 | 0.001◊ | 49.1 ± 13.4 | 19.1 ± 2.6 | <0.001◊ | 48.8 ± 15.7 | 32.3 ± 16.2 | <0.001◊ |
| Range | 8 – 60 | 14 – 60 | 14 – 60 | | 22 - 60 | 14 - 25 | | 20 -60 | 14 – 60 | |
| **DFS** | | | | | | | | | | |
| Mean ± SD | 29.4 ± 17.1 | 51.7 ± 12 | 20.9 ± 12.9 | <0.001◊ | 37.4 ± 14.6 | 11.4 ± 1.6 | <0.001◊ | 39 ± 17.9 | 21.3 ± 11.5 | <0.001◊ |
| Range | 114- 60 | 2 – 60 | 8 – 49 | | 12 - 60 | 8 - 15 | | 10 -60 | 8 – 38 | |

*P* < 0.05 is statistically significant; #Chi square test ◊Mann Whitney test; Notch-4: Neurogenic locus notch homology 4; SATB-2: Special AT rich sequence binding protein 2; DFS: Disease-free survival, OS: overall survival

## Availability of Data and Materials

The datasets used and/or analyzed during the current study are available from the authors upon reasonable request.

## Conflict of Interest

None declared.

## References


1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin.* 2018;68(6):394-424. doi: 10.3322/caac.21492. Erratum in: *CA Cancer J Clin*. 2020;70(4):313.
2. Gado A, Ebeid B, Abdelmohsen A, Axon A. Colorectal cancer in Egypt is commoner in young people: is this cause for alarm? *Alex J Med.* 2014;50(3):197–201. doi: 10.1016/j.ajme.2013.03.003.
3. Aran V, Victorino AP, Thuler LC, Ferreira CG. Colorectal cancer: epidemiology, disease mechanisms and interventions to reduce onset and mortality. *Clin Colorectal Cancer.* 2016;15(3):195-203. doi: 10.1016/j.clcc.2016.02.008.
4. Annett S, Robson T. Targeting cancer stem cells in the clinic: Current status and perspectives. *Pharmacol Ther.* 2018; 187:13-30. doi: 10.1016/j.pharmthera.2018.02.001.


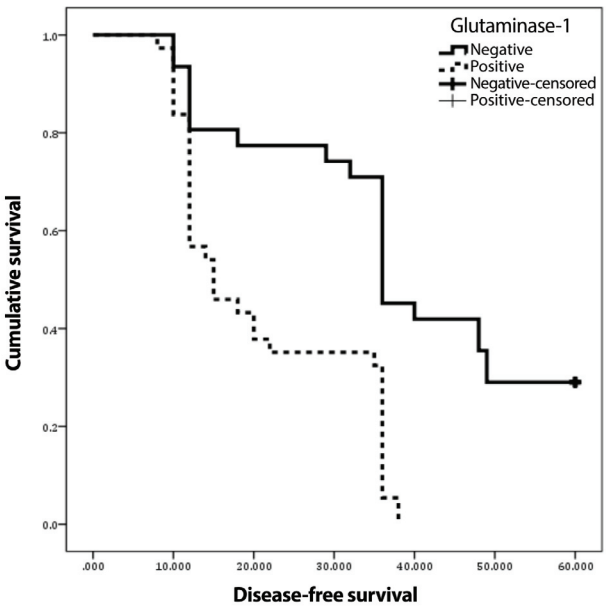


**Figure 11.** Kaplan-Meier plot showing a significant relationship between Glutaminase-1 and disease-free survival.

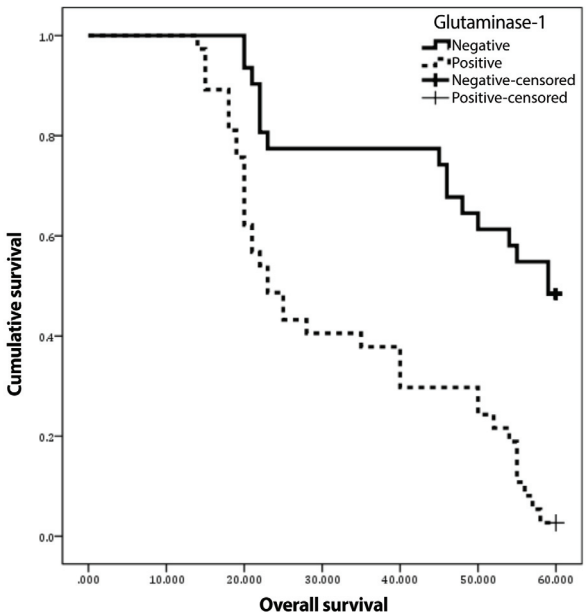


**Figure 12.** Kaplan-Meier plot showing the relationship between Glutaminase-1 and overall survival.

5. Aster JC, Pear WS, Blacklow SC. The varied roles of Notch in cancer. *Annu Rev Pathol.* 2017;12:245-75. doi: 10.1146/annurev-pathol-052016-100127.
6. Nowell CS, Radtke F. Notch as a tumour suppressor. *Nat Rev Cancer.* 2017;17(3):145-159. doi: 10.1038/nrc.2016.145.
7. Berg KB, Schaefer DF. SATB2 as an immunohistochemical marker for colorectal adenocarcinoma a concise review of benefits and pitfalls. *Arch Pathol Lab Med.* 2017;141(10):1428–33. doi: 10.5858/arpa.2016-0243-RS.
8. Hrudka J, Matěj R, Nikov A, Tomyak I, Fišerová H, Jelínková K, et al. Loss of SATB2 expression correlates with cytokeratin 7 and PD-L1 tumor cell positivity and aggressiveness in colorectal cancer. *Sci Rep.* 2022;12(1):19152. doi: 10.1038/s41598-022-22685-0.
9. Guo C, Xiong D, Yao X, Gu W, Zhang H, Yang B, et al. Decreased SATB2 expression is associated with metastasis and poor prognosis in human clear cell renal cell carcinoma. *Int J Clin Exp Pathol.* 2015;8(4):3710-8.
10. Ma C, Olevian DC, Lowenthal BM, Jayachandran P, Kozak MM, Chang DT, et al. Loss of SATB2 expression in colorectal carcinoma is associated with DNA mismatch repair protein deficiency and BRAF mutation. *Am J Surg Pathol.* 2018;42(10):1409-17. doi: 10.1097/PAS.0000000000001116.
11. Dum D, Kromm D, Lennartz M, De Wispelaere N, Büscheck F, Luebke AM, et al. SATB2 expression in human tumors. *Arch Pathol Lab Med.* 2023; 147(4): 451-64.doi:10.5858/arpa.2021-0317-OA.
12. Zhang J, Pavlova NN, Thompson CB. Cancer cell metabolism: the essential role of the nonessential amino acid, glutamine. *EMBO J.* 2017;36(10):1302-15. doi: 10.15252/embj.201696151.
13. Tanaka K, Sasayama T, Irino Y, Takata K, Nagashima H, Satoh N, et al. Compensatory glutamine metabolism promotes glioblastoma resistance to mTOR inhibitor treatment. *J Clin Invest.* 2015;125(4):1591-602. doi: 10.1172/JCI78239.
14. Pan T, Gao L, Wu G, Shen G, Xie S, Wen H, et al. Elevated expression of glutaminase confers glucose utilization via glutaminolysis in prostate cancer. *Biochem Biophys Res Commun.* 2015; 456(1):452-8. doi: 10.1016/j.bbrc.2014.11.105.
15. Gross MI, Demo SD, Dennison JB, Chen L, Chernov-Rogan T, Goyal B, et al. Antitumor activity of the glutaminase inhibitor CB-839 in triple-negative breast cancer. *Mol Cancer Ther*. 2014;13(4): 890-901. doi: 10.1158/1535-7163.MCT-13-0870.
16. Zhang Z, Bu X, Yang J, Zhu S, He S, Zheng J, et al. NOTCH4 regulates colorectal cancer proliferation, invasiveness, and determines clinical outcome of patients. *J Cell Physiol.* 2018;233(10):6975-85. doi: 10.1002/jcp.26619.
17. Lin F, Shi J, Zhu S, Chen Z, Li A, Chen T, et al. Cadherin-17 and SATB2 are sensitive and specific immunomarkers for medullary carcinoma of the large intestine. *Arch Pathol Lab Med*. 2014; 138(8):1015-26. doi: 10.5858/arpa.2013-0452-OA.
18. Xu L, Zhou D, Li F, Ji L. Glutaminase 2 functions as a tumor suppressor gene in gastric cancer. *Transl Cancer Res.* 2020;9(8):4906-13. doi: 10.21037/tcr-20-2246.
19. Bonyadi Rad E, Hammerlindl H, Wels C, Popper U, Ravindran Menon D, Breiteneder H, et al. Notch4 signaling induces a mesenchymal-epithelial-like transition in melanoma cells to suppress malignant behaviors. *Cancer Res.* 2016;76(7):1690-7. doi: 10.1158/0008-5472.CAN-15-1722.
20. Wu G, Chen Z, Li J, Ye F, Chen G, Fan Q, et al. NOTCH4 Is a Novel prognostic marker that correlates with colorectal cancer progression and prognosis. *J Cancer.* 2018;9(13):2374-9. doi: 10.7150/jca.26359.
21. Fukusumi T, Guo TW, Sakai A, Ando M, Ren S, Haft S, et al. The NOTCH4-HEY1 pathway induces epithelial-mesenchymal transition in head and neck squamous cell carcinoma. *Clin Cancer Res.* 2018;24(3):619-33. doi: 10.1158/1078-0432.CCR-17-1366.
22. Mansour MA, Hyodo T, Ito S, Kurita K, Kokuryo T, Uehara K, et al. SATB2 suppresses the progression of colorectal cancer cells via inactivation of MEK5/ERK5 signaling. *FEBS J.* 2015;282(8):1394-405. doi: 10.1111/febs.13227.
23. Dragomir A, de Wit M, Johansson C, Uhlen M, Pontén F. The role of SATB2 as a diagnostic marker for tumors of colorectal origin: Results of a pathology-based clinical prospective study. *Am J Clin Pathol.* 2014;141(5):630-8. doi: 10.1309/AJCPWW2URZ9JKQJU.
24. Zhang YJ, Chen JW, He XS, Zhang HZ, Ling YH, Wen JH, et al. SATB2 is a promising biomarker for identifying a colorectal origin for liver metastatic adenocarcinomas. *EBioMedicine.* 2018;28:62-9. doi: 10.1016/j.ebiom.2018.01.001.
25. Mezheyeuski A, Ponten F, Edqvist PH, Sundström M, Thunberg U, Qvortrup C, et al. Metastatic colorectal carcinomas with high SATB2 expression are associated with better prognosis and response to chemotherapy: a population-based Scandinavian study. *Acta Oncol.* 2020;59(3):284-90. doi: 10.1080/0284186X.2019.1691258.
26. Luo LJ, Yang F, Ding JJ, Yan DL, Wang DD, Yang SJ, et al. MiR-31 inhibits migration and invasion by targeting SATB2 in triple negative breast cancer. *Gene.* 2016;594(1):47-58. doi: 10.1016/j.gene.2016.08.057.
27. Yang MH, Yu J, Chen N, Wang XY, Liu XY, Wang S, et al. Elevated microRNA-31 expression regulates colorectal cancer progression by repressing its target gene SATB2. *PLoS One.* 2013;8(12):e85353. doi:

10.1371/journal.pone.0085353.
28. Xiang L, Mou J, Shao B, Wei Y, Liang H, Takano N, et al. Glutaminase 1 expression in colorectal cancer cells is induced by hypoxia and required for tumor growth, invasion, and metastatic colonization. *Cell Death Dis.* 2019;10(2):40. doi: 10.1038/s41419-018-1291-5.
29. Huang F, Zhang Q, Ma H, Lv Q, Zhang T. Expression of glutaminase is upregulated in colorectal cancer and of clinical significance. *Int J Clin Exp Pathol.* 2014;7(3):1093–100.
30. Song Z, Wei B, Lu C, Li P, Chen L. Glutaminase sustains cell survival via the regulation of glycolysis and glutaminolysis in colorectal cancer. *Oncol Lett*. 2017;14(3):3117-23. doi: 10.3892/ol.2017.6538.