REVIEW
SATB1 and 2 in colorectal cancer
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Abstract
The special AT-rich sequence-binding proteins 1 and 2 (SATB1/2) are nuclear matrix associated proteins that are transcription factors involved in chromatin remodeling and gene regulation. Expression of the SATB2 gene is tissue-specific, and the only epithelial cells expressing SATB2 are the glandular cells of the lower gastrointestinal tract where its expression is regulated by microRNA-31 (miR-31) and miR-182. SATB2, along with its homolog SATB1, are thought to be involved in various cancers with their roles in this disease being specific to the type of cancer. Colorectal cancer (CRC) provides the largest association of SATB2 with cancer and the roles of SATB2 are better defined and more studied in CRC than in any other cancer type. SATB1 displays a negative association with SATB2 in CRC. The various studies that have investigated the involvement of SATB1 and 2 in CRC have produced consistent findings. Here, we form four major conclusions regarding the role of these proteins in CRC and their potential clinical value: (i) SATB2 is a sensitive marker to distinguish CRC from other cancer types, (ii) Reduced expression of SATB2 in CRC is associated with poor prognosis, (iii) High levels of SATB1 expression facilitate CRC and are associated with poor prognosis and (iv) Overexpression of miR-31 and -182 in CRC leads to more aggressive cancer. This review will describe several of the key investigations that established these conclusions and highlight results that offer opportunities for future research in the treatment and diagnosis of CRC.

Introduction
The special AT-rich sequence-binding proteins 1 and 2 (SATB1/2) are nuclear matrix-associated proteins that are important for growth and development (1,2). Since the discovery of SATB2 in 2003 (3), there has been a rapid increase in studies investigating the roles of this protein (2,4-6). SATB2 gene was discovered in a complementary DNA subtraction screening in a search for genes controlling neural differentiation (1). First identified as a putative cleft palate gene and named as a homolog of the DNA matrix binding protein SATB1, SATB2 consists of 11 exons spanning 191 kilobases on chromosome 2 that encodes a 733 amino acid protein with a predicted molecular weight of 82.5 kDa. The protein has two CUT domains (amino acids 352–437) and one homeodomain (amino acids 614–677 (3)). SATB2 is highly conserved across vertebrate species from zebrafish to chickens to mammals (7). SATB2 expression is tissue-specific, and the only epithelial cells expressing this protein in adult tissue are the glandular cells lining the lower gastrointestinal (GI) tract (1).

SATB2 is involved in the differentiation of neurons (4), stem cells (8), and osteoblasts (2). SATB2 was first discovered as a transcription factor that plays a crucial role in the regulation of bone development and differentiation of osteoblasts (2). It is thought to be involved in chromatin remodeling by recruiting histone acetyltransferases and deacetyltransferases to chromatin for the regulation of gene expression. It was found that SATB2 forms a complex with HDAC1 in vitro and in vivo (6).

MicroRNAs (miRNAs), as well as distant regulatory elements, have been proven to play a significant role in controlling the expression of SATB2. For example, AS021, a short interspersed repetitive sequence (SINE), which is a type of retrotranspon
that may act as a distal enhancer resulting in an increase in SATB2 expression (9). SATB2 is negatively regulated by miRNA 31 and 182 [miR-31 and miR-182 (10,11)]. RunX2, a transcription factor essential for osteoblastogenesis, blocks the miR-23a–27a–24-2 cluster, which negatively regulate SATB2 mRNA (12). Moreover, RunX2 is a negative regulator of miR-31 (13) and thus indirectly increases SATB2 levels. Furthermore, the SATB2 protein undergoes a post-translational modification by PIA1, which adds one or two SUMO groups on either lysine 233 or 350. This modification directs the protein to the nuclear periphery and decreases its ability to enhance transcription of downstream target genes (14). Dysfunctions in these regulators have been shown to perturb SATB2 levels in normal and cancerous tissue (10,11,15). SATB2 regulation can vary by cell type; however, miR-31 seems to be the most common regulator of SATB2 amongst cell types.

Similar to SATB2, SATB1 is a nuclear matrix-associated protein that acts in chromatin remodeling mechanisms to regulate the expression of multiple genes (16–18). SATB1 is primarily expressed in thymocytes (19). This protein folds chromatin into loops and recruits chromatin remodeling and modifying enzymes to these DNA loops. Depending on the genomic location, SATB1 attracts histone deacetylases and other histone modifying enzymes to the site and a change in histone marks occur that may either activate or repress transcription (16,20). SATB1 is involved in cell differentiation by opening the chromatin structure around cell-type specific genes to allow for transcription factors and chromatin remodeling proteins to bind. SATB1 interacts with the Wnt-b-catenin pathway to facilitate thymocyte development (21,22) and is involved with the activation of Th2 cells by inducing an upregulation of the gene GATA3 (22).

SATB1 expression is also associated with many types of cancers, colorectal (18), breast (23), pancreatic (24), nasopharyngeal (25), bladder (26), prostate (27), lung (28), ovarian (29), liver (30), glioma (31), lymphoma (32) and kidney (33). Table 1 outlines the major associations of SATB1 in different cancer types. Many of the genes that are regulated by SATB1 have roles in carcinogenesis, ERRB2, ABL1, MMP2, E-CADHERIN, VEGFB, TGFB1 and KISS1 (16,17). A study by Han et al. (51) demonstrated that SATB1 promotes breast cancer metastasis and progression by altering the expression of over 1000 genes associated with breast cancer. SATB1 expression in most cancers is associated with progression and poor prognosis (23,25,26).

SATB2 has also been increasingly linked to cancer. Colorectal (52), head and neck (53) and bone (54) cancers have been associated with SATB2 expression. The various reports regarding the involvement of SATB2 in different cancers (Table 2) demonstrate a cancer-specific role of SATB2. Colorectal cancer (CRC) provides the largest association of SATB2 and cancer. The roles of SATB2 are more defined and more studied in CRC than in any other cancer type. The effects of SATB2 expression on prognosis and metastasis are different in CRC than any other cancer type. SATB1 is also involved in CRC, and its effects on progression and prognosis are negatively associated with SATB2. SATB1’s role in CRC seems to be in line with its involvement in other cancers. This review will discuss the four major findings regarding SATB1/2 in CRC studies. To our knowledge, this is the first review summarizing SATB1/2’s role in CRC.

### SATB2 and CRC

CRC is the third leading cause of death due to cancers, and over 1 million cases are identified each year (59). Although CRC is curable at early stages, it is characterized by rapid progression and delayed onset of clinical symptoms (60). The many studies that have investigated the involvement of SATB1 and 2 in CRC have produced consistent results, and several conclusions can be established regarding the role of these proteins in CRC and their clinical use: (i) SATB2 is a sensitive marker to distinguish

### Table 1. SATB1 and Cancer

<table>
<thead>
<tr>
<th>Cancer type</th>
<th>Conclusions</th>
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<tr>
<td>CRC</td>
<td>1. Expression increases tumor progression and metastasis.</td>
<td>(18,34–36)</td>
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<td></td>
<td>2. Expression provides for poor prognosis.</td>
<td>(23,37,38)</td>
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<td></td>
<td>3. Correlates with a loss of SATB2 expression.</td>
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<tr>
<td>Breast</td>
<td>1. Expression increases metastasis.</td>
<td>(24)</td>
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<td></td>
<td>2. Expression provides for poor prognosis.</td>
<td>(25,39–41)</td>
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<td>Pancreatic cancer</td>
<td>1. Associated with decreased survival but an increased sensitivity to chemotherapy.</td>
<td>(26,42)</td>
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<tr>
<td>Nasopharyngeal carcinoma</td>
<td>1. Overexpression of SATB1 is an independent prognostic factor for NPC and aids in progression of the tumor.</td>
<td>(27,43)</td>
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<tr>
<td>Bladder</td>
<td>1. Upregulated in bladder cancer and promotes metastatic features.</td>
<td>(28,44)</td>
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<tr>
<td>Prostate</td>
<td>1. Expression appears in metastasis.</td>
<td></td>
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<td></td>
<td>2. Induces aggressive tumors by facilitating the epithelial-to-mesenchymal transition (EMT).</td>
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<td>Lung</td>
<td>1. Loss of expression provides for poor prognosis in squamous cell carcinomas (SCC).</td>
<td>(29,45,46)</td>
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<tr>
<td></td>
<td>2. Treatment with trichostatin-A, a histone deacetylase inhibitor, recovered SATB1 levels in SCC cells.</td>
<td>(30,47,48)</td>
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<td></td>
<td>3. Upregulated in small cell lung cancer (SCLC) tissue and is associated with proliferation and invasion in SCLC cells.</td>
<td>(31,49)</td>
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<tr>
<td>Ovarian</td>
<td>1. Expression is a factor of poor prognosis and metastasis.</td>
<td>(32,50)</td>
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<tr>
<td>Liver</td>
<td>1. SATB1 is involved in the development and progression of liver cancer.</td>
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<td></td>
<td>2. Expression is associated with recurrence and metastasis.</td>
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<tr>
<td>Glioma</td>
<td>1. Expression associated with poor prognosis.</td>
<td>(33)</td>
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<td>Lymphoma</td>
<td>1. SATB1 aids in lymphoma by influencing X-IST-induced silencing.</td>
<td></td>
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<tr>
<td></td>
<td>2. SATB1 becomes upregulated via DNA demethylation of its promoter and silences p21 in lymphoma.</td>
<td>(34)</td>
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<tr>
<td>Kidney</td>
<td>1. Overexpression of SATB1 provides for an aggressive tumor phenotype by inducing EMT in renal cell carcinoma.</td>
<td>(35)</td>
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CRC from other cancer types, (ii) Reduced expression of SATB2 in CRC is associated with poor prognosis, (iii) High levels of SATB1 expression facilitate CRC and are associated with poor prognosis and (iv) Overexpression of miR-31 and -182 in CRC leads to more aggressive cancer. The following sections will review several of the key investigations that these conclusions were based on.

**SATB2 as a biomarker for CRC**

Expression of SATB2 is primarily preserved in CRC and is a highly sensitive marker to distinguish CRC from other cancer types. A study by Magnusson et al. aimed to explore the specific expression pattern of SATB2 in relation to a well-known biomarker of CRC, cytokeratin 20 (CK20), by examining tumors from nine cohorts (n = 1882) of patients with primary and metastatic CRCs. The results showed that 85% of all CRCs were positive for SATB2 and 97% for SATB2 and/or CK20. Other cancer types examined in the study were found to rarely display SATB2 expression, including cancers of the upper GI tract. Ovarian cancer is an important diagnosis to differentiate from CRC; in the non-CRC cohorts, ovarian cancers did not display SATB2 expression (55). The clinical importance of SATB2 as a biomarker of CRC increases when comparing medullary carcinoma of the large intestine, which is negative for many established CRC biomarkers such as CK20, from other cancer types. Lin et al. found that 89% of medullary carcinomas were positive for SATB2, whereas 97% of total CRCs were positive for SATB2 expression. Consistent with Magnusson et al., the majority of other cancer types tested displayed very low or undetectable expression of SATB2; however, lymphoid cells did display significant levels of SATB2, which offers a potential pitfall in the use of SATB2 as a biomarker for tumors with an increased level of lymphoid cells (61).

Other potential pitfalls include the metal-induced increase of SATB2. The expression of SATB2 is uniformly increased in cells transformed by carcinogenic metals, nickel, arsenic and chromium [VI (62)], suggesting that SATB2 induction is involved in metal carcinogenesis. Upregulation of SATB2 in metal-induced tumors from other organs besides the lower GI tract may generate false-positive results when diagnosing CRC by detection of SATB2. For example, arsenic increased SATB2 expression in human bronchial epithelial cells (62), and arsenic exposure causes lung cancer (63); therefore, it is probable that arsenic-induced lung tumors may also display increased SATB2 expression. Studies should be conducted to evaluate SATB2 expression in tumors from cohorts with occupational or high exposure to nickel, arsenic and chromium (VI) in order to consider metal-induced tumors when diagnosing CRC based on SATB2. Other CRC biomarkers, such as cadherin-17, that are not known to be affected by metals should be used in conjunction with SATB2; reduced MLH1 would not be a candidate since it is also repressed by chromium (VI) exposure (64).

CRC can be cured if it is detected in the early stages or if optimal adjuvant treatment can be identified. However, very few molecular markers have been identified to select patients for adjuvant therapy, and these biomarkers often display poor abilities for clinical use. There is an urgency to identify valid biomarkers for CRC detection and prognosis. Studies have demonstrated the high sensitivity and specificity of SATB2 to distinguish CRC from other cancer types, and the potential use of SATB2 in the clinic warrants further investigations. Given the chance for false-positive results, a panel of proteins including CK20, CK7, MLH1, cadherin-17 and SATB2 should be considered as a diagnostic tool for CRC.

**SATB2 expression in CRC and prognosis**

Various epidemiological studies have concluded that SATB2 expression in CRC provides for a better prognosis than SATB2 negative CRCs. Eberhard et al. demonstrated that overexpression of SATB2 in CRC increased survival and enhanced the patient’s benefit from adjuvant therapy. Microsatellite instability (MSI), which is often seen in CRC and can render 5-FU treatment ineffective, was negatively associated with SATB2 expression (56).

The Magnusson study reported that cases of metastasis displayed lower levels of SATB2 expression compared with primary tumors (55) and another study showed that low SATB2 expression in CRC is associated with metastasis, tumor invasion and metastasis of lymph nodes (52). These results suggest that loss of SATB2 expression may be involved in the progression of CRC by facilitating features associated with metastasis. These studies support the Lin study, which demonstrated that SATB2 expression was reduced in medullary carcinoma of the large intestine compared with other CRC types (61). Further studies are required to uncover the mechanisms linking SATB2 overexpression with good prognosis for CRC. Favorable prognosis may not be in a causative relationship with SATB2, but instead the association may occur due to an increase of negative regulators of SATB2 that facilitate cancer progression through other mechanisms.

**SATB1 overexpression in CRC**

SATB1 overexpression has a negative association with SATB2 and confers poor prognosis for CRC. A study by Nodin et al. (18) found that SATB1 expression was positively associated with MSI and overexpression of β-catenin and is a factor of poor prognosis in SATB2-negative tumors. The correlation of SATB1 expression with MSI and poor prognosis is consistent with findings by the Eberhard study described above. Another study by Zhang et al. found no correlation between SATB1 and SATB2 and demonstrated that SATB1 expression was associated with shorter survival time in both SATB2-negative and -positive CRC cases. An in vitro experiment by the same group revealed that knockdown

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**Table 2. SATB2 and Cancer**

<table>
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<th>Cancer type</th>
<th>Major conclusions</th>
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| CRC         | 1. Expression is correlated with good prognosis.  
             | 2. Loss of expression is due to upregulation of miR-31 and -182 and provides for poor prognosis.  
             | 3. Loss of expression is correlated with SATB1 expression. | (10,11,35,55,56,57) |
| Bone        | 1. SATB2 enhances migration and invasion in osteosarcoma.  
             | 2. SATB2 is a marker of osteoblastic differentiation in benign and malignant mesenchymal tumors. | (5,37) |
| Head and neck | 1. SATB2 acts as a tumor suppressor in laryngeal squamous cell carcinoma wherein loss of expression was associated with recurrence and high tumor grade.  
               | 2. In head and neck squamous cell carcinoma, SATB2 expression provides for resistance to chemotherapy. | (36,58) |
of SATB1 repressed colony formation, migration, and invasion and increased apoptosis in a CRC cell line. Opposite effects were observed for each endpoint when SATB1 was overexpressed in the same cell line (34,65).

SATB1 expression levels were found to be associated with tumor differentiation and stage in an investigation by Zhang et al. (65) SATB1 levels are higher in poorly differentiated compared with well-differentiated CRC cells. Other markers, such as NFκB and cyclin D1, that increase cell proliferation were correlated positively with SATB1 protein levels. Several other investigations have demonstrated the association of SATB1 expression in CRC and poor prognosis (35,36). In contrast to these reports, a study by Al-Sohaily et al. (66) found that SATB1 expression was associated with increased survival but CRC patients without SATB1 expression still responded better to adjuvant therapy.

The above studies point toward the prognostic potential of SATB1, as well as implicate it as a possible therapeutic target. Unlike SATB2 expression, the normal colorectal mucosa contains very low to undetectable levels of SATB1 (18). The onset of carcinogenesis probably maintains or increases SATB2 levels, whereas SATB1 stays repressed until later stages. The processes that lead to negative associations with SATB1 and 2 on prognosis are currently unexplained, and future studies should investigate possible interactions between the two proteins. One hypothesis is that high SATB2 levels overcome the carcinogenic potential provided by the initial increase in SATB1. As the cancer progresses, SATB2 expression decreases directly by miR-31 and -182 expression or other possible indirect mechanisms, which provides for the effects of SATB1 to become apparent. The aberrant increase in SATB1 expression is unexplained but one study provides a possible mechanism. Wang et al. found that expression of the prion PrPc correlated with poor prognosis of CRC based on histological examinations. Knockdown of PrPc resulted in loss of SATB1 expression and reduced metastatic capacity in CRC cells (67). Targeting PrPc in chemotherapy to combat the carcinogenic potential of SATB1 in CRC should be considered.

miRNA expression in CRC

The connection between SATB2 expression and prognosis is best explained by miR-31 expression in CRC. Several investigations have reported an association between increased levels of miR-31 and CRC cases with poor prognosis (10,57), which coincide with results (52, 55,56) that report increased SATB2 expression provides for favorable prognosis. As mentioned earlier, miR-31 targets SATB2 transcripts, and ectopic expression of miR-31 depletes SATB2 mRNA and protein levels. A study by Yang et al. showed that overexpression of miR-31 in CRC cells increased the cells’ ability to proliferate, invade and migrate, whereas inhibition of miR-31 reduced the cells’ aggressive phenotype. The metastatic features induced by miR-31 were found to be mediated by an increase in molecular markers of the epithelial-to-mesenchymal transition (EMT). When cells overexpressing miR-31 were inoculated in nude mice, the group found that miR-31 overexpression enhanced tumor growth, invasion and metastasis. To investigate whether SATB2 depletion was associated with increased metastatic features in miR-31 overexpressed cells, they overexpressed SATB2 without a 3’-untranslated sequence in miR-31 overexpressed cells. SATB2 overexpression was sufficient to reduce the invasion and migratory ability of the cells. In the human component of the study, the group found that CRC patients with tumors overexpressing miR-31 were associated with aggressive phenotypes and poor prognosis (10). Consistent with these results, Schee et al. (57) found that high expression of miR-31 was associated with advanced tumor stage and poor differentiation.

miR-31 is not the only miRNA that targets SATB2 in CRC. Several investigations have identified miR-182 as a negative regulator of SATB2 expression and reported its increase in CRC (68–70). Similar to miR-31, increased expression of miR-182 is correlated with poor prognosis (11,69).

An in-depth study by Yang et al. investigated the mechanisms behind increased miR-182 expression and CRC progression. Subjects with metastasis displayed higher levels of miR-182 than subjects without metastasis. Overexpression of miR-182 in CRC cells resulted in increased proliferation, ability to form colonies in soft agar, invasiveness and migratory abilities; the increase in these carcinogenic endpoints was reversed when a miR-182 inhibitor was added. The group also found increased tumors and metastasis in vivo when mice were injected with overexpressed miR-182 CRC cells. Loss of SATB2 was found to be a key event in miR-182-induced progression of CRC. Restoration of SATB2 levels by transfection in miR-182 overexpressing reduced cell proliferation, invasion and migration. Loss of SATB2 by miR-182 increased the mesenchymal markers, Snail and Vementin, and decreased E-cadherin, an epithelial marker; increased levels of SATB2 reversed the results for all three markers. Earlier we stated that SATB2 forms a complex with HDAC1 and recruits chromatin remodelers. The SATB2/HDAC1 complex may remodel the chromatin structure that regulates the SNAIL gene to reduce expression of SNAIL and prevent EMT (11).

miR-31 and miR-182 upregulation facilitates CRC progression and is an unfavorable prognostic factor. Very few studies have reported on the mechanisms behind miR-31 and miR-182-induced CRC progression, but the major findings have pointed towards SATB2 being a key mediator. It is probably that miR-31 and -182 influence CRC through several mechanisms, given their ability to target multiple mRNAs. In addition to CRC, miR-31 is upregulated in head and neck squamous cell carcinoma (71), hepatocellular carcinoma (72), squamous cell carcinoma of the tongue (73) and lung cancer (74), and its upregulation has been found to positively impact development and/or progression of these cancers. In addition to CRC, miR-182 is upregulated in ovarian cancer (75). In contrast to the findings in CRC, miR-31 is downregulated in invasive urothelial carcinoma of the bladder (76), prostate cancer (77) and breast cancer (78). These findings indicate that miR-31 and -182 have tumor-specific roles. In breast cancer, miR-31 expression inhibits breast cancer metastasis (78) and miR-182 expression sensitizes breast cancer cells to chemotherpy with PARP inhibitors by decreasing BRCAl1 levels (79). miR-31 is upregulated in lung cancer (74), whereas miR-182 expression suppresses lung tumorigenesis (80). The diverse results regarding miR-31 and -182 in cancer allude to the involvement of many different pathways and molecules that mediate miR-31 and -182’s carcinogenic potential. Several of these other target genes include DNA repair genes. A study by Lynam-Lennon et al. (81) found that miR-31 overexpression down-regulated 11 DNA repair genes. Similarly, miR-182 targets about 1000 genes, and especially disrupts the homologous recombinational repair pathway (82). Perturbations of DNA repair pathways lead to genomic instability, which is a consistent feature of cancer cells. Future studies on CRC should investigate other targets of miR-31 and -182 and examine possible interactions with SATB2.

Conclusion

Metastasis of CRC cells to other organs is responsible for the majority of CRC deaths (59,60). However, mechanisms underlying
molecular events leading to metastasis in CRC are unclear and require further investigations. Studies of SATB1/2 hold potential in unraveling these mechanisms; SATB2 is a tumor-suppressor in CRC and can attenuate miR-31 and -182-mediated malignant phenotypes in CRC. SATB2 has been shown to be a sensitive biomarker for CRC, and expression is associated with good prognosis. As CRC progresses and becomes metastatic, SATB2 expression is lost and thus, may not be a sensitive detection method for late stage CRC. Loss of SATB2 is accompanied by an increase miR-31 and miR-182, both of which are unfavorable prognostic factors. SATB1 expression is lost in advanced stages of CRC and its detection represents aggressive phenotypes of CRC and poor survival. SATB1 holds potential as a biomarker for later stages of the disease; however, it must be used in conjunction with other biomarkers specific for CRC.

In addition to CRC, SATB1 and 2 are involved in several other cancers but their roles and influence on prognosis vary by cancer type. Tables 1 and 2 outline the major conclusions found regarding SATB1/2 in different cancer types. The general consensus for most cancers is that SATB1/2 positively regulate carcinogenic endpoints such as invasion, migration, tumor grade, recurrence and metastasis, while providing for poor prognosis. Several cancers have shown exceptions to this trend. For SATB2, CRC and laryngeal squamous cell carcinoma display opposite results, whereas squamous cell carcinoma of the lung shows different outcomes for SATB1. The unique ability of SATB2 to decrease cancer progression in CRC may be related to its tissue-specific expression wherein the only epithelial cells expressing this protein are the glandular cells lining the lower GI tract (1). Investigations examining the normal functions of SATB2 in the intestinal epithelia may shed light on its distinctive role in CRC.

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