A COMPARISON OF SATB2 EXPRESSION RESULTS WITH CK20 AND CDX2 IN METASTATIC COLORECTAL CARCINOMA CASES

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ABSTRACT

Introduction*: SATB2 (The special AT-rich sequence-binding protein 2) is the new DNA binding protein and nuclear transcription factor. In normal epithelial tissues, the SATB2 protein is specifically expressed in the nuclei of the lower gastrointestinal (GI) tract epithelial cells. The elective expression of SATB2 in the lower GI tract implies that it can be used as a diagnostic marker for metastatic colorectal carcinoma (CRC).*

Materials and methods*: Immunohistochemical (IHC) analysis was performed by applying SATB2, CK20 and CDX2 in 96 metastatic CRC cases (51 liver, 22 lung, 19 ovarian, 3 skin and 1 uterine myometrial tissue) with various origins. The aim is to evaluate the diagnostic value of SATB2 alone, and the double and triple combinations of SATB2 with CK20 and CDX2.*

Results*: Out of 96 metastatic colon carcinoma cases, 94 (97.9%) had positive SATB2 expressions. It was found that 93.7% (90/96) of the cases had expression with CK 20 and CDX2. The sensitivity of SATB2 alone was 97.92%, and the sensitivity of CK20 and CDX2 alone was the same, i.e. 93.75%. This rate was also higher in the double combination of SATB2 with CDX2 and CK20 in comparison to the double combination of CK20 and CDX2. The sensitivity of SATB2 and CK20 combination was 98.8%, which was higher than that of CK20 and CDX2 combination.*

Conclusions*: Our results support the requirement of including SATB2 in the IHC panel for metastatic CRC cases.*

Key words*: CDX2, CK20, colorectal carcinoma metastasis, SATB2.*

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Introduction

Colorectal carcinoma (CRC) is a globally important health concern due to its high prevalence and mortality rates. Every year, 1 million individuals are diagnosed with CRC and more than 500,000 patients die from it[(1)].

An estimated 3-5% of all cancer cases clinically manifest with a metastasis of an unknown primary tumor[(2)]. In metastatic cases that morphologically mimic primary tumors, a suitable immunohistochemical (IHC) panel is selected for the diagnosis based on the histological morphology of the tumor[(3,4)]. However, the primary focus can not be identified in a majority of the cases, although IHC methods are commonly used[(5)].

The selection of antibodies that recognize the target proteins is of the utmost importance while selecting the IHC panel. However, very few of these antibodies, which are routinely used for pathology, are expressed in specific cell types. The prostate specific antigen that marks the prostate glandular cells, thyroglobulin that marks the thyroid glandular cells, and the glial fibrillary acidic protein that marks astrocytes are some of these antibodies[(3)].

On the other hand, a majority of antibodies have limited diagnostic specificity since they are expressed in more than one cell type. The most commonly used IHC markers in metastatic tumors that originate from the colon consist of Cytokeratin20 (CK20) and Caudal type homeobox 2 (CDX2)[(3,4,6)].

But these markers do not have high specificity because both can be expressed in tumors other than colon carcinoma. CK20 can also be expressed in urothelial epithelial and epidermal Merkel cells in addition to gastrointestinal system tumors[7]. CDX2 can also be positive in gastric carcinoma and ovarian mucinous tumors. Diffuse expression is detected in 85-100% of the metastatic cases of colonic origins with CK20[8,9] and in 61-100% of the cases with colon carcinoma with CDX2[10,11].

SATB2 (The special AT-rich sequence-binding protein 2) is the new DNA binding protein and nuclear transcription factor with a length of 733 amino acids. SATB2 is associated with gene transcription and chromatin remodeling. Previous studies demonstrated that SATB2 plays an important role in brain development, craniofacial modelling and osteoblast differentiation[12].

IHC studies showed that SATB2 is strongly expressed not only in the normal and neoplastic osteoblastic tissue, but also in the normal colorectal and appendiceal epithelia[13].

In normal epithelial tissues, the SATB2 protein is specifically expressed in the nuclei of the lower gastrointestinal (GI) tract epithelial cells. It is also shown that the SATB2 protein was expressed in non-epithelial cell types (in some lymphoid cells, testicular germ cells and some neurons in the central nervous system). The elective expression of SATB2 in the lower GI tract implies that it can be used as a diagnostic marker for colorectal carcinoma. Therefore, this potential diagnostic biomarker has been analyzed in many CRC and other cancer types[14].

This study investigates the SATB2, CK20 and CDX2 expression results in 96 cases with CRC metastasis. The aim is to evaluate the diagnostic value of SATB2 alone, and the double and triple combinations of SATB2 with CK20 and CDX2.

Materials and methods

Cases whose biopsy results were reported to be consistent with CRC metastasis in the Pathology Departments of Gaziantep and Inonu Universities between January 2007 and June 2018 were included in this study. All patient preparations who have a clinical history of CRC or those who were confirmed to have colon cancer after the diagnosis were reviewed by two pathologists.

In total, 96 metastatic CRC cases comprised of 51 liver, 22 lung, 19 ovarian, 3 skin tissues and 1 uterine myometrial tissue were included in the study. Paraffin blocks of the preparations that contained the largest epithelial tumor component were selected, and IHC analysis was performed by applying SATB2, CK20 and CDX2 on the sections prepared from these blocks.

The Ethical Committee's approval according to the principles of the Helsinki declaration was awarded for this study.

Assessment of immunoreactivity

The IHC antibodies SATB2 (rabbit monoclonal antibody EP281, Cell Marque, USA), CK20 (rabbit monoclonal antibody SP33, Ventana, USA) and CDX2 (rabbit monoclonal antibody EPR2764Y, Cell Marque, USA) were studied using an automated immunohistochemistry-staining device (Ventana, Bench Mark Ultra Auto-Stainer, USA).

In the IHC assessment of the SATB2 expression, preparations were evaluated in terms of expression intensity and the extensiveness of expression in the tumor cells. The colon adenocarcinoma case that also contained normal colon epithelia was used as an external control.

Based on the expression intensity in the colon epithelia which was used as external control, the staining in the tumor area was assessed as weak (1), moderate (2) and strong (3). The extensiveness of expression in the tumor tissue was scored between 0-4. Accordingly, if less than 1% of the tumor cells, 2-25%, 26-50%, 51-75% of the tumor cells and more than 75% of the tumor cells had expression, the extensiveness of expression was scored as 0, 1(+), 2(+), 3(+) and 4(+), respectively. A final score ranging between 0-12 was given according to the multiplication of the extensiveness and intensity of expression in the tumor[6].

The scoring was performed by two pathologists independent of each other. Both pathologists obtained the same results in 90% of cases. For the remaining cases, the final score was given according to their shared decision.

The expression's presence was accepted as positive for CK20 and CDX2 regardless of the intensity and extensiveness[3].

As a result, the nuclear staining for SATB2 and CDX2, and cytoplasmic staining for CK20 were accepted as positive if more than 1% of tumor cells were positive. Normal colon tissue was used as a positive control.

Statistical examinations

Descriptive statistics were provided in numbers and percentage. The relationships between categorical variables were determined using the Chi-square test. SPSS 22.0 package software was used for the analyses. P<0.05 was accepted as significant.

Results

Out of 96 metastatic colon carcinoma cases, 94 (97.9%) had positive SATB2 expressions. Among the positive cases, 80 (85.1%) had strong, 12 (12.8%) had moderate and 2 (2.1%) had weak SATB2 expressions. 91 (96.8%) of the positive cases had 4(+) positivity (extensiveness higher than 75%). Among the 2 negative cases, 1 had liver metastasis and the other had lung metastasis.

It was found that 93.7% (90/96) of the cases had expression with CK20 and CDX2.

The results for SATB2, CK20 and CDX2 expression are summarized in Table 1 and expression patterns in different tissues are showed in Figure1.

Table 1: Results of SATB2, CK20 and CDX2 separately and for double and triple combinations

	CRC metastasis (n:96)	Sensitivity (%)	PPV (%)	Accuracy (%)	Youden index
SATB2 +	94	97.92	100	97.92	0.979
CK20 +	90	93.75	100	93.75	0.937
CDX2 +	90	93.75	100	93.75	0.937
SATB2 +/ CK20 +	89	98.89	100	93.75	0.156
SATB2 +/ CDX2 +	88	97.78	93.62	91.67	-0.022
CK20 +/ CDX2 +	86	95.56	95.56	91.67	0.289
All three positive	85	88.54	100	88.54	0.885
Any of two positive	93	96.87	100	96.87	0.968
Any of one positive	96	100	100	100	1

Discussion

Since the discovery of SATB2 in 2003 , there has been a rapid increase in the number of studies that investigate the role of this protein[15]. The latest data shows that SATB2 expression behaves like a tumor suppressor gene in various tumor types[16-18]. Mansour et al. revealed that SATB2 behaved like a tumor suppressor gene in colorectal cancer through ERK5 inactivation. In addition, they also showed that low expression or lack of expression of SATB2 was a sign of malignant behavior and poor prognosis in CRC[19].

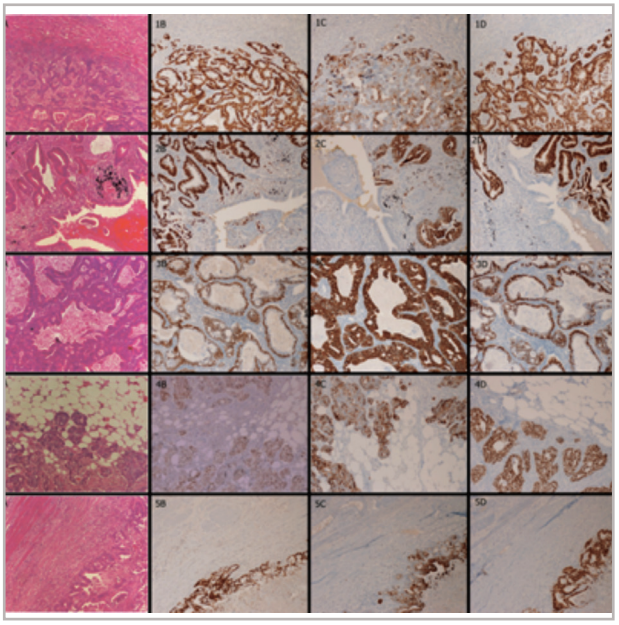

Fig. 1: The histological morphology and the immunohistochemical expression of SATB2, CK20 and CDX2 in metastatic colorectal carcinoma cases.
1A: H&E section of metastatic CRC in liver. **1B**: Diffuse and strong nuclear SATB2 expression of tumor in liver. **1C**: Cytoplasmic CK20 expression of tumor in liver. 1D: Nuclear CDX2 expression of tumor in liver .
2A: H&E section of metastatic CRC in lung . **2B**: Diffuse and strong nuclear SATB2 expression of tumor in lung **2C**: Cytoplasmic CK20 expression of tumor in lung .**2D**: Nuclear CDX2 expression of tumor in lung .
3A: H&E section of metastatic CRC in ovary. **3B**: Diffuse and moderate nuclear SATB2 expression of tumor in ovary. **3C**: Cytoplasmic CK20 expression of tumor in ovary. **3D**: Nuclear CDX2 expression of tumor in ovary.
4A: H&E section of metastatic CRC in subcutaneous tissue. 4B: Diffuse and moderate nuclear SATB2 expression of tumor in subcutaneous tissue. **4C**: Cytoplasmic CK20 expression of tumor in subcutaneous tissue. **4D**: Nuclear CDX2 expression of tumor in subcutaneous tissue.
5A: H&E section of metastatic CRC in uterin myometrium. **5B**: Diffuse and moderate nuclear SATB2 expression of tumor in uterin myometrium. **5C**: Cytoplasmic CK20 expression of tumor in uterin myometrium. **5D**: Nuclear CDX2 expression of tumor in uterin myometrium.
All representative H&E and IHC samples at x100 magnification are shown.

In other studies, it was found that SATB2 expression was associated with a good prognosis in CRC cases[20,21].

There are studies indicating that SATB2 could be used in the diagnosis of CRC metastases after the studies that support the idea that SATB2 is a specific and sensitive marker of CRC[3,4,6,14,21-23].

CK20 and CDX2 are commonly used markers in the diagnosis of metastatic CRC cases. This current study aimed to assess the presence of SATB2 expression in metastatic CRC cases and to compare the results with CK20 and CDX2 expression results in order to determine the most suitable combination that can be used in the diagnosis of metastatic CRC cases.

Our study's results found that SATB2 sensitivity was 97.9% in cases studies that consisted of 96 metastatic CRC cases with different localizations of the CRC. This result, which supports the importance of SATB2 in diagnosis, was higher than many series in the literature.

In a study by Magnusson et al. that consisted of 9 independent cohorts of 1,882 patients and employed the tissue microarray method, it was found that SATB2 was expressed in 85.8% of primary CRC cases and in 205 (81.3%) of 252 cases included in the metastatic patient group. Based on these results, it was shown that SATB2 was a sensitive and extremely specific marker for primary and metastatic CRC. The mentioned rate was found to reach 97% as a result of the combination of SATB2 and CK20[14].

In the series of Dragomir et al. that contained colorectal and non-colorectal (lung, gynecologic, pancreatobiliary, renal, prostatic and gastroesophageal) carcinoma cases, it was found that SATB2 had 93% sensitivity and 77% specificity in supporting colorectal origin[3].

In the series of Zhang et al. that contained 97 metastatic CRC cases compared with other tumors that metastasized to the liver, the sensitivity and specificity of SATB2 alone was found to be 92.2% and 97.8%, respectively. It was observed that the sensitivity rate was 89.3% and 92.2% in combination with CK20 and CDX2, respectively and the same rate reached 97.8% in the combination of three[6].

In this current study, the sensitivity of SATB2 alone was 97.92%, which is higher in comparison to CK 20 and CDX2. The sensitivity of CK20 and CDX2 alone was the same, i.e. 93.75%. This rate was also higher in the double combination of SATB2 with CDX2 and CK20 in comparison to the double combination of CK20 and CDX2. The sensitivity of SATB2 and CK20 combination was 98.8%, which was higher than that of SATB2 and CDX2 combination. These results support the requirement of including SATB2 in the IHC panel for CRC metastasis. However, the fact that a high rate of CDX2 and SATB2 positivity is also seen in ovarian mucinous tumors[4] and that a high rate of CK20 and SATB2 positivity is also seen in tumors that originate from the appendix limits the use of both combinations alone[24].

Consequently, all these results indicate that the diagnostic value of the triple combination of SATB2, CK20 and CDX2 will be high.

The fact that this study only used metastatic CRC cases and did not make any comparisons with other metastatic carcinomas is considered as a limiting factor. However, this current study does not contain a limited amount of tissue like the tissue microarray method, and it enables the assessment of expression in the entire tumor tissue. In addition, the studied series did not target only one metastatic organ as the results from the other organs were evaluated as well.

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