



# The frequency of SATB2 and CDX2 expression in metastatic mucinous tumors of ovarian and colonic origin

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## Abstract

**Introduction:** Metastatic mucinous tumors present a unique diagnostic challenge for surgical pathologists due to their varied origins and potential diagnostic difficulties.

**Objectives:** This study aimed to determine the frequency of SATB2 and CDX2 expression in metastatic mucinous tumors of ovarian and colonic origin.

**Materials and Methods:** Paraffin blocks (n = 40) containing mucinous tumors of ovarian or colonic origin were collected from two educational hospitals affiliated with Isfahan University of Medical Sciences between 2009 and 2019. Prepared slides were stained for special AT-rich sequence-binding protein 2 (SATB2) and caudal type homeobox 2 (CDX2). The nuclear intensity of SATB2 or CDX2 staining was graded as follows: "0" for negative, "1+" for weak, "2+" for moderate, and "3+" for strong. The percentage of tumor staining was graded based on three categories: the majority of tumor staining (>50%) as "2", minority of tumor staining (5-49%) as "1", and negative staining (<5%) as "0". The final histologic score (H-score) was calculated by combining these intensity and percentage scores.

**Results:** Both SATB2 and CDX2 were more frequently expressed in metastatic mucinous tumors of colonic origin compared to ovarian origin (65.5% versus 36.4% and 93.1% versus 72.7%, respectively), but the difference was not statistically significant. No significant differences were observed between metastatic mucinous tumors of colonic and ovarian origin regarding SATB2 and CDX2 nuclear intensity, percentage of tumor staining, and H-score. The sensitivity and specificity of CDX2 for differentiating between colonic and ovarian origin were 93.10% and 27.27%, respectively. The corresponding diagnostic values for SATB2 were 65.52% and 63.64%, in sequence.

**Conclusion:** Neither of the two biomarkers, nor their combinations, demonstrated acceptable diagnostic values for distinguishing between metastatic mucinous tumors of ovarian and colonic origin.

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## Introduction

Since metastatic mucinous tumors' morphology and clinical features often pose diagnostic challenges for surgical pathologists (1), accurately identifying their primary origins becomes crucial. These tumors can arise as primary lesions from various sources, such as the breast, lung, upper gastrointestinal, and gynecologic tracts (1). It is important to note that mucinous malignancies exhibit significant overlap in their immunophenotypic features across different primary sites (2).

The caudal type homeobox 2 (CDX2) is a transcription factor that is predominantly localized within the nucleus of intestinal epithelial cells. It has been observed to exhibit non-specific staining in various primary mucinous tumors (3). Extensive research on mucinous tumors of ovarian origin has shown CDX2 expression ranging from 26

## Key point

- The diagnosis of metastatic mucinous tumors poses pathological challenges due to their varied morphology.
- CDX2 and SATB2 are primarily expressed in metastatic mucinous tumors.
- CDX2 and SATB2 demonstrate low diagnostic value in distinguishing between ovarian and colonic origin in metastatic mucinous tumors.

to 79% (4). In contrast, the special AT-rich sequence-binding protein 2 (SATB2) has emerged as a more specific marker for colorectal tumors (5). SATB2 is primarily expressed in the glandular epithelium of the lower gastrointestinal tract and serves as an indicator for metastatic mucinous colorectal adenocarcinomas, particularly when compared to ovarian mucinous neoplasms (6-8).

## Objectives

Considering these factors, the objective of this study was to determine the frequency of SATB2 and CDX2 expression in metastatic mucinous tumors originating from the ovaries and colon. Additionally, the diagnostic value of these markers in distinguishing between the two origins was evaluated.

## Materials and Methods

### Participants and study design

The study included a total of 40 paraffin blocks of mucinous tumors collected and stored at Al-Zahra and Shahid Beheshti hospitals in Isfahan, Iran, between 2009 and 2019. The inclusion criteria involved selecting paraffin blocks with sufficient tissue for evaluation, hematoxylin-eosin (H&E) stained slides, and samples containing at least 50% mucinous components.

Firstly, the age and gender of the patients corresponding to the tumor blocks were recorded. For SATB2 staining, formalin-fixed blocks containing paraffin-embedded tissue were cut into 4-5  $\mu\text{m}$  sections and placed on positively charged slides. The slides were then incubated at 60 °C for 60 minutes and subjected to discovery cell conditioner #1 at 95 °C for 36 minutes. Subsequently, SATB2 specific antibody (manufactured by Zytomed, Germany) was applied at a dilution of 1:25. The slides were further incubated at 37 °C for 16 minutes, and the antibody was detected using an antibody detector. The antibody was then assessed through an antibody detector. CDX2 staining was performed using a similar protocol. The blocks were cut into sections and placed on slides, followed by incubation at 60 °C for 60 minutes. The slides were then subjected to discovery cell conditioner #1 at 100 °C for 8 minutes (repeated three times). CDX2 specific antibody (manufactured by Biocare, USA) was applied at a dilution of 1:1000. After incubation at 37°C for 32 minutes, all slides were evaluated by a single pathologist.

The nuclear intensity of SATB2 or CDX2 staining was assessed and graded as follows: “0” for negative staining, “1+” for weak staining, “2+” for moderate staining, and “3+” for strong staining, in comparison to the intensity of SATB2 and CDX2 staining in normal colon controls (2). Additionally, the percentage of tumor staining was categorized into three groups: “2” for the majority of tumor staining (>50%), “1” for the minority of tumor staining (5-49%), and “0” for negative staining (<5%).

To calculate the final histologic score (H-score), the intensity and percentage scores were added together. A score of “0” indicated a total absence of intensity/percentage staining, while a score of “5” indicated the maximum intensity/percentage staining (2).

### Statistical analysis

Data analysis was conducted using the Statistical Package for the Social Sciences (SPSS) software (version 25.0,

Armonk, NY: IBM Corp., USA). Mean  $\pm$  Standard Deviation was used to describe quantitative variables, while frequencies and percentages were conducted for qualitative variables. The Fisher's exact test was applied to compare qualitative variables between metastatic tumors of ovarian and colonic origin. A *P* value of  $\leq 0.05$  was considered statistically significant. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and diagnostic accuracy (DA) of the markers were estimated for colonic and ovarian origin separately.

## Results

In the current study, a total of 40 blocks of metastatic mucinous tumors, originating from either the colon or the ovary, were examined. The average age of the patients associated with these tumor blocks was  $59.08 \pm 14.00$  years. Among these patients, 16 (40%) were male, while 24 (60%) were female. Out of the metastatic tumors analyzed, 11 (27.5%) originated from the ovary, whereas 29 (72.5%) were of colonic origin.

The expression of SATB2 was detected in 23 (57.5%) cases, while CDX2 was expressed in 35 (87.5%) cases. Table 1 presents the frequency of CDX2 and SATB2 expression based on the origin of the metastatic tumor. Although both SATB2 and CDX2 were more frequently expressed in metastatic mucinous tumors originating from the colon compared to those originating only from the ovary (65.5% versus 36.4% and 93.1% versus 72.7%, respectively), this difference was not found to be statistically significant. Furthermore, no significant differences were observed between metastatic mucinous tumors originating from the colon and those originating from the ovary in terms of SATB2 and CDX2 nuclear intensity, percentage of tumor staining, and H-score (Table 1).

Table 2 demonstrates that the highest sensitivity (93.10%), NPV (41.18%), and DA (75.00%) were achieved when considering a positive result for CDX2 or at least one positive marker for differentiating the colonic origin of metastatic mucinous tumors from their ovarian origin. On the other hand, the highest specificity (63.64%) and PPV (82.61%) were observed when considering a positive result for SATB2 or both positive markers (Table 2).

In contrast, when differentiating the ovarian origin of metastatic mucinous tumors, the highest sensitivity (72.73%) and PPV (22.86%) were achieved when considering a positive result for CDX2 or at least one positive marker. Conversely, the highest specificity (34.48%), NPV (58.82%), and DA (35.00%) were observed when considering a positive result for SATB2 or both positive markers (Table 3).

## Discussion

In the current study, we observed comparable positivity of CDX2 and SATB2 in metastatic mucinous tumors with colonic and ovarian origins. Furthermore, there were no

**Table 1.** Frequency of SATB2 and CDX2 expression, nuclear intensity, percentage of tumor staining, and H-score by tumor origin

Variables	Ovary (n=11)	Colon (n=29)	P value*
<b>SATB2</b>			
Positive, N (%)	4 (36.4)	19 (65.5)	0.153
Nuclear intensity, N (%)			
0	7 (63.6)	10 (34.5)	0.154
1	0 (0.0)	7 (24.1)	
2	0 (0.0)	3 (10.3)	
3	4 (36.4)	9 (31.0)	
Percentage of tumor staining, N (%)			
0	7 (63.6)	10 (34.5)	0.295
1	0 (0.0)	3 (10.3)	
2	4 (36.4)	16 (55.2)	
H-score, N (%)			
0	7 (63.6)	10 (34.5)	0.357
2	0 (0.0)	3 (10.3)	
3	0 (0.0)	4 (13.8)	
4	0 (0.0)	3 (10.3)	
5	4 (36.4)	9 (31.0)	
<b>CDX2</b>			
Positive, N (%)	8 (72.7)	27 (93.1)	0.117
Nuclear intensity, N (%)			
0	3 (27.3)	2 (6.9)	0.406
1	1 (9.1)	3 (10.3)	
2	1 (9.1)	5 (17.2)	
3	6 (54.5)	19 (65.5)	
Percentage of tumor staining, N (%)			
0	3 (27.3)	3 (10.3)	0.412
1	2 (18.2)	4 (13.8)	
2	6 (54.5)	22 (75.9)	
H-score, N (%)			
0	3 (27.3)	2 (6.9)	0.340
1	0 (0.0)	1 (3.4)	
2	1 (9.1)	1 (3.4)	
3	1 (9.1)	2 (6.9)	
4	0 (0.0)	4 (13.8)	
5	6 (54.5)	19 (65.5)	

Abbreviations: SATB2, Special AT-rich sequence-binding protein 2; CDX2, Caudal type homeobox 2.

\* Analyzed by the Fisher's exact test.

significant differences between tumors of these origins in terms of nuclear intensity, percentage of tumor staining, and H-score for both SATB2 and CDX2. However, we did find that strong SATB2 nuclear intensity and an H-score of

5 were more frequently observed in metastatic mucinous tumors with ovarian origin, while a higher percentage of tumor staining was more common in tumors with colonic origin. Additionally, the frequencies of CDX2 expression were higher in mucinous tumors with colonic origin. These findings are consistent with the results reported by Brettfeld et al, who also found higher frequencies of strong SATB2 nuclear intensity and percentage of tumor staining in primary colorectal mucinous tumors (2).

Another important finding of the current study was that positive SATB2 values were always accompanied by positive CDX2 results, indicating a strong correlation between the two markers. In other words, there were no cases of positive SATB2 accompanied by negative CDX2. This finding is consistent with the study conducted by Salim et al, who reported that all positive SATB2 cases were also positive for CDX2 (9). Salim et al evaluated cases of already-diagnosed metastatic carcinoma with diverse origins and reported a high sensitivity of SATB2 expression for colorectal cancer, which aligns with the investigation conducted by Magnusson et al (10).

However, it is important to note that one of the important results of our study was the relatively low SATB2 sensitivity for tumors of colonic origin. This indicates that SATB2 may not be as sensitive in detecting colonic origin compared to other markers or in comparison to its sensitivity for other origins.

The value of SATB2 in differentiating metastatic mucinous tumors has been extensively studied. Montiel et al concluded that SATB2 is a useful biomarker for determining whether mucinous intestinal-type ovarian tumors are primary or metastatic. They found that SATB2 was highly sensitive in detecting metastases from the lower gastrointestinal tract (8). Similarly, Moh et al demonstrated that ovarian tumors with mucinous or endometrioid characteristics expressing SATB2 had a primary ovarian origin (6).

However, Ramos et al showed that SATB2 was a less sensitive indicator of colorectal origin when comparing mucinous colorectal carcinoma to conventional ones. They also reported that SATB2 exhibited considerably lower specificity in mucinous gastroesophageal primary tumors (11). On the other hand, Hewedi et al reported that compared to CDX2, SATB2 was a more sensitive but less specific marker for differentiating mucinous carcinoma of colorectal origin from those of other origins (12).

**Table 2.** Diagnostic value of SATB2 and CDX2 for the detection of colonic origin of metastatic mucinous tumor

Markers	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	DA (%)
SATB2 positive	65.52	63.64	82.61	41.18	65.00
CDX2 positive	93.10	27.27	77.14	60.00	75.00
Both positive	65.52	63.64	82.61	41.18	65.00
At least one positive	93.10	27.27	77.14	60.00	75.00

Abbreviations: SATB2, Special AT-rich sequence-binding protein 2; CDX2, Caudal type homeobox 2; PPV, Positive predictive value; NPV, Negative predictive value; DA, Diagnostic accuracy.

**Table 3.** Diagnostic value of SATB2 and CDX2 for the detection of ovarian origin of metastatic mucinous tumor

Markers	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	DA (%)
SATB2 positive	36.36	34.48	17.39	58.82	35.00
CDX2 positive	72.73	6.89	22.86	40.00	25.00
Both positive	36.36	34.48	17.39	58.82	35.00
At least one positive	72.73	6.89	22.86	40.00	25.00

Abbreviations: SATB2, Special AT-rich sequence-binding protein 2; CDX2, Caudal type homeobox 2; PPV, Positive predictive value; NPV, Negative predictive value; DA, Diagnostic accuracy.

In the current study, the highest sensitivity was observed with CDX2 for the detection of mucinous metastatic tumors with colonic origin. However, CDX2, CK7, and CK20 had limited sensitivity and specificity for distinguishing metastatic from primary ovarian mucinous tumors (13-17). These findings suggest that while SATB2 may be a useful biomarker in certain contexts, such as distinguishing primary and metastatic mucinous tumors in the ovaries, its sensitivity and specificity may vary depending on the specific tumor types and origins being evaluated.

Although the current study exclusively discussed metastatic mucinous tumors originating from the ovaries and colon, it is important to acknowledge the limitations that were encountered. These limitations include the relatively small sample size and the restriction in generalizability of the findings. As a result, it is crucial to interpret the results of this study with caution.

### Conclusion

In conclusion, while CDX2 exhibited high sensitivity in differentiating the colonic origin of metastatic mucinous tumors from their ovarian origin, its specificity was relatively low. Overall, neither of the two markers nor any combination of them had acceptable diagnostic values for distinguishing between the ovarian and colonic origin of metastatic mucinous tumors.

### Limitations of the study

There were certain limitations in this study, including the sample size and the restriction of generalizability. Therefore, the results should be interpreted with caution.

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### Authors' contribution

**Conceptualization:** Pegah Hedayat.

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### Conflicts of interest

The authors confirm that they have no competing interests.

### Ethical issues

The research conducted in this study adhered to the principles outlined in the Declaration of Helsinki and was approved by the Ethics Committee of Isfahan University of Medical Sciences (Ethical code#IR.MUI.MED.REC.1399.616). Prior to any intervention, all participants provided written informed consent. This paper was extracted from the pathology residency thesis of Razieh Ghasemi (Thesis #399564) at this university. The authors addressed ethical concerns, including plagiarism, data fabrication, and double publication.

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### References

1. Chu PG, Chung L, Weiss LM, Lau SK. Determining the site of origin of mucinous adenocarcinoma: an immunohistochemical study of 175 cases. *Am J Surg Pathol.* 2011;35:1830-6. doi: 10.1097/PAS.0b013e3182299c25.
2. Brettfield SM, Ramos BD, Berry RS, Martin DR, Hanson JA. SATB2 Versus CDX2: A Battle Royale for Diagnostic Supremacy in Mucinous Tumors. *Arch Pathol Lab Med.* 2019;143:1119-1125. doi: 10.5858/arpa.2018-0337-OA.
3. Bayrak R, Haltas H, Yenidunya S. The value of CDX2 and cytokeratins 7 and 20 expression in differentiating colorectal adenocarcinomas from extraintestinal gastrointestinal adenocarcinomas: cytokeratin 7-/20+ phenotype is more specific than CDX2 antibody. *Diagn Pathol.* 2012;7:9. doi: 10.1186/1746-1596-7-9.
4. Li Z, Roth R, Rock JB, Lehman A, Marsh WL, Suarez A, et al. Dual Immunostain With SATB2 and CK20 Differentiates Appendiceal Mucinous Neoplasms From Ovarian Mucinous Neoplasms. *Am J Clin Pathol.* 2017;147:484-491. doi: 10.1093/ajcp/axq023.
5. Berg KB, Schaeffer DF. SATB2 as an Immunohistochemical Marker for Colorectal Adenocarcinoma: A Concise Review of Benefits and Pitfalls. *Arch Pathol Lab Med.* 2017;141:1428-1433. doi: 10.5858/arpa.2016-0243-RS.
6. Moh M, Krings G, Ates D, Aysal A, Kim GE, Rabban JT. SATB2 Expression Distinguishes Ovarian Metastases of Colorectal and Appendiceal Origin From Primary Ovarian Tumors of Mucinous or Endometrioid Type. *Am J Surg Pathol.* 2016;40:419-32. doi: 10.1097/PAS.0000000000000553.
7. Strickland S, Wasserman JK, Giassi A, Djordjevic B, Parra-Herran C. Immunohistochemistry in the Diagnosis of



- Mucinous Neoplasms Involving the Ovary: The Added Value of SATB2 and Biomarker Discovery Through Protein Expression Database Mining. *Int J Gynecol Pathol.* 2016;35:191-208. doi: 10.1097/PGP.0000000000000238.
8. Perez Montiel D, Arispe Angulo K, Cantú-de León D, Bornstein Quevedo L, Chanona Vilchis J, Herrera Montalvo L. The value of SATB2 in the differential diagnosis of intestinal-type mucinous tumors of the ovary: primary vs metastatic. *Ann Diagn Pathol.* 2015;19:249-52. doi: 10.1016/j.anndiagpath.2015.05.004.
  9. Salim S, Asif M, Ahmed R, Din HU, Khadim MT, Sadaf G. Diagnostic utility of SATB2 in metastatic colorectal carcinoma. *PAFMJ.* 2021;71:S32-6. doi: 10.51253/pafmj.v71iSuppl-1.3034.
  10. Magnusson K, de Wit M, Brennan DJ, Johnson LB, McGee SF, Lundberg E, et al. SATB2 in combination with cytokeratin 20 identifies over 95% of all colorectal carcinomas. *Am J Surg Pathol.* 2011;35:937-48. doi: 10.1097/PAS.0b013e31821c3dae.
  11. Ramos BD, Brettfeld S, Berry RS, Routh JK, Martin DR, Hanson JA. A Comprehensive Evaluation of Special AT-rich Sequence-binding Protein 2 (SATB2) Immunohistochemical Staining in Mucinous Tumors From Gastrointestinal and Nongastrointestinal Sites. *Appl Immunohistochem Mol Morphol.* 2019;27:378-385. doi: 10.1097/PAI.0000000000000627.
  12. Hewedi IH, Shakweer MM, Radwan NA. Diagnostic value of the combined use of SATB2 and CDX2 in mucinous carcinoma of colorectal origin. *Egyptian J Pathol.* 2017;37(1):112-9. doi: 10.1097/01.XEJ.0000516726.57921.51.
  13. Jagirdar J. Application of immunohistochemistry to the diagnosis of primary and metastatic carcinoma to the lung. *Arch Pathol Lab Med.* 2008;132:384-96. doi: 10.5858/2008-132-384-AOITTD.
  14. Logani S, Oliva E, Arnell PM, Amin MB, Young RH. Use of novel immunohistochemical markers expressed in colonic adenocarcinoma to distinguish primary ovarian tumors from metastatic colorectal carcinoma. *Mod Pathol.* 2005;18:19-25. doi: 10.1038/modpathol.3800260.
  15. Saad RS, Ghorab Z, Khalifa MA, Xu M. CDX2 as a marker for intestinal differentiation: Its utility and limitations. *World J Gastrointest Surg.* 2011;3:159-66. doi: 10.4240/wjgs.v3.i11.159.
  16. Shin JH, Bae JH, Lee A, Jung CK, Yim HW, Park JS, et al. CK7, CK20, CDX2 and MUC2 Immunohistochemical staining used to distinguish metastatic colorectal carcinoma involving ovary from primary ovarian mucinous adenocarcinoma. *Jpn J Clin Oncol.* 2010;40:208-13. doi: 10.1093/jjco/hyp150.
  17. Vang R, Gown AM, Wu LS, Barry TS, Wheeler DT, Yemelyanova A, et al. Immunohistochemical expression of CDX2 in primary ovarian mucinous tumors and metastatic mucinous carcinomas involving the ovary: comparison with CK20 and correlation with coordinate expression of CK7. *Mod Pathol.* 2006;19:1421-8. doi: 10.1038/modpathol.3800698.