Differential Diagnostic Value of SATB2 in Primary Pulmonary Enteric Adenocarcinoma and Pulmonary Metastases of Colorectal Carcinoma

Jingping Yuan

Renmin Hospital of Wuhan University: Wuhan University Renmin Hospital

Lin Xiong

Renmin Hospital of Wuhan University: Wuhan University Renmin Hospital

Ying Zhou

Renmin Hospital of Wuhan University: Wuhan University Renmin Hospital

Juan Luo

Renmin Hospital of Wuhan University: Wuhan University Renmin Hospital

Huihua He

Renmin Hospital of Wuhan University: Wuhan University Renmin Hospital

Honglin Yan

Renmin Hospital of Wuhan University: Wuhan University Renmin Hospital

Li Xu (✉ 925580164@qq.com)

Renmin Hospital of Wuhan University: Wuhan University Renmin Hospital

Research

Keywords: SATB2, PEA, colorectal carcinomas, CK7

Posted Date: December 4th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-119067/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Aims:

Pulmonary enteric adenocarcinoma (PEA) is composed mainly of tall columnar cells arranged in an irregular acinar or cribriform pattern with extensive central necrosis, closely resembling the appearance of intestinal epithelial and colorectal carcinomas under the microscope. PEA is usually positive for CK7. However, some cases lack CK7 expression and are positive for intestinal differentiation markers, such as CDX2, villin, and CK20. It is difficult to distinguish between PEA and pulmonary metastases of colorectal carcinoma (MCRC), so new identification methods need to be explored.

Methods and Results:

We found that SATB2 was highly tissue-specific by bioinformatics analysis. It is highly expressed in intestinal tissues, and its expression level in lung tissues is very low. Then, according to the WHO diagnostic criteria, the cases of lung tumor were preliminary screened to obtain PEA and MCRC cases. In the preliminary screening process, immunohistochemical staining of common lung adenocarcinoma immunomarkers (CK7, TTF-1, and NapsinA) and common intestinal cancer immunomarkers (CK20, CDX2, and villin) were performed to aid identification. Then, after combined with clinical history, imaging examination and colonoscopy, 28 primary PEA specimens and 42 MCRC specimens were final included. The above process was independently reviewed by two pathologists. PEA and MCRC had differences in the number of tumor nodules. The positive rates of SATB2 in PEA and MCRC were 0.00% and 92.86%, respectively. The sensitivity, specificity and diagnostic accuracy of SATB2 for distinguishing MCRC from PEA were 92.86%, 100% and 95.71%, respectively. The diagnostic accuracy of CK7 was 88.57%, which was slightly inferior to SATB2, and the results of CK7 could be used as a reference for SATB2 differential diagnosis.

Conclusions:

Our study showed that SATB2 can be viewed as the best immunomarkers for distinguishing PEA from MCRC. At the same time, CK7 could be used as references for SATB2 differential diagnosis.

1. Introduction

Primary enteric adenocarcinoma (PEA) is a rare histological subtype of lung adenocarcinoma. PEA is mainly composed of tall columnar cells arranged in an irregular acinar or cribriform pattern with extensive central necrosis, closely resembling the appearance of intestinal epithelial and colorectal carcinomas under the microscope[1]. Therefore, it is difficult to distinguish between PEA and pulmonary metastases of colorectal carcinoma (MCRC) by morphology.

CK20 is an intermediate filament protein that is selectively expressed in glandular cells of the gastrointestinal (GI) tract. CK20 is stably expressed in colorectal cancer, and the positive rate is greater

than 90%. It also retains positive characteristics in metastasis[2]. However, the specificity of CK20 alone is relatively low because this keratin is also expressed in the gastric epithelium, urothelium, and epidermal Merkel cells[3]. CDX2 is a highly sensitive immunomarker for gastrointestinal (GI) adenocarcinomas but may also be expressed in tumors from other organs, such as the pancreas, bile ducts, bladder, uterine cervix, endometrium, and ovary[4]. Numerous previous studies reported that CK7 was positively expressed in almost all PEA patients, but the lack of CK7 expression was also reported in a small number of patients with PEA[5, 6]. CK7 is one of the cytokeratin subtypes and is mainly expressed in glandular epithelial cells. The positive expression rate of CK7 in colorectal cancer tissues was 10%~27%[7]. So, in clinical practice, CK20 is often combined with other markers, such as the presence of CDX2 or the absence of CK7, to improve the accuracy of the diagnosis of colorectal carcinoma (CRC). However, some PEA cases lack CK7 expression and are positive for intestinal differentiation markers, such as CDX2, villin, and CK20[8]. In this case, the combined diagnosis of immune indicators cannot also get accurate results.

As mentioned above, the morphology of PEA and MCRC are similar, and it is also difficult to make a differential diagnosis with commonly used immunomarkers. However, the lung presents a common site of metastasis for colorectal carcinoma, and the treatment strategies and prognosis outcomes differ considerably for patients with these malignancies[9]. So, it is of vital importance for pathologists and clinicians to make a clear distinguish between these entities. Therefore, we need to find new differential diagnosis methods to distinguish between the two types of cancer.

The special AT-rich sequence binding-protein (SATB2) is a DNA-binding protein, which contains 733 amino acids and specifically binds to nuclear matrix attachment regions of DNA. SATB2 expression is tissue-specific, and the only epithelial cells expressing this protein in adult tissues are the glandular cells lining the lower GI tract[10]. SATB2 is a protein involved in transcription regulation and shows nuclear staining, thus, it could have some advantages over cytoplasmic/membrane staining markers such as cytokeratin. In a recent study, SATB2 was detected in more than 1,800 cases of CRC and more than 600 cases of other tumors. The results suggested that SATB2 was highly specific for primary and metastatic lesions of CRC, and showed a similar level of sensitivity to CRC as CK20, CDX2, and CDH17. Besides, SATB2 in combination with CK20 can identify more than 95% of all CRC[11]. The above studies indicate that SATB2 may provide valuable information for clinical decision-making to confirm or exclude colorectal-derived tumors. In view of the high sensitivity and specificity of SATB2 for the pathological diagnosis of primary and metastatic CRC, it can be used to distinguish colorectal cancer from other cancers. This study collected 28 primary PEA specimens and 42 MCRC specimens to detect the specificity and sensitivity of SATB2 in PEA and MCRC, and to explore its differential diagnostic value in the two types of carcinoma.

2. Materials And Methods

2.1 Bioinformatics analysis

The expression level of SATB2 in different tissues was searched by pubmed (<https://pubmed.ncbi.nlm.nih.gov/>). In an article[12] with biological project number PRJEB4337, RNA sequencing was performed in tissue samples from 95 individuals representing 27 different tissues. We got RNA information of SATB2 from this article.

2.2 Patients and clinicopathological factors

After obtaining approval from the Ethical Committee of Renmin Hospital of Wuhan University (WDRY2019-K010), we preliminary screened the samples of patients with lung tumor who were admitted to Renmin Hospital of Wuhan University from January 2015 to June 2020 according to the WHO diagnostic criteria[1]. The specimens were independently reviewed by two pathologists, and immunohistochemical staining of common lung adenocarcinoma immunomarkers (CK7, TTF-1, and NapsinA) and common intestinal cancer immunomarkers (CK20, CDX2, and villin) was performed to aid identification. Finally, after carefully analyzing clinical history, lower abdomen CT and colonoscopy, we recruited 28 primary PEA specimens and 42 MCRC specimens. For cases that are still difficult to diagnose, follow up on the latest clinical diagnosis for at least 6 months to determine the final diagnosis. All patients provided signed informed consent.

The clinicopathological data of all patients were collected from the department of pathology and department of thoracic surgery. These clinicopathological data include age, gender, tumor size, tumor number, smoking history, pleura invasion, bronchus invasion, lymph node metastasis, vessel invasion and nerve invasion.

2.3 Immunohistochemical staining

All surgically resected specimens were fixed with 10% buffered formalin. We selected a representative slide of each case. After pretreatment with PT-link (Dako), immunohistochemistry analysis was performed using the Autostainer Link 48 (Dako).

Primary antibodies against cell markers used in this study included SATB2, CK20, CK7, CDX2, villin, TTF-1, NapsinA, MLH1, MSH2, MSH6 and PMS2, which were purchased from DAKO (Carpinteria, CA, USA). The dilution concentration of the antibodies is 1:1000. A medium-intensity colored tissue was used as a positive control, and PBS was used as a negative control instead of the primary antibody. As a result, it was determined that the positive cells were accurately positioned to be effective.

2.4 Staining Evaluation

We use the binary classification method to evaluate the immunostaining results. For CK7, CK20, villin, NapsinA, TTF-1 and CDX-2, tumors were considered positive if staining was found in $\geq 10\%$ of the neoplastic cells. For SATB2, because the immunostains of SATB2 in PEA specimens all showed negative, while the immunostains of SATB2 in MCRC specimens almost all showed strong positive. We used the 2-tiered scoring (all or nothing) method to evaluate the immunostains of SATB2. Tumor cells were considered to be positive for SATB2 when brownish-yellow nuclear particles were seen in tumor cells.

For MLH1, PMS2, MSH2, and MSH6, preserved expression was defined as nuclear staining within tumor cells, using infiltrating lymphocytes as a positive internal control. Loss of MLH1, PMS2, MSH2, and MSH6 protein expression was defined as the complete absence of nuclear staining in tumor cells with concurrent positive labeling in internal non-neoplastic tissues. Proficient MMR protein expression was defined as preserved nuclear expression of all four MMR proteins. Deficient MMR protein expression was defined as loss of protein expression of at least one of the 4 MMR proteins.

2.5 Sensitivity, specificity and diagnostic accuracy

Sensitivity, specificity, and diagnostic accuracy were calculated to assess test performance. The formula for the calculation is as follows:

Sensitivity = true positive number / (true positive number + false negative number) × 100%

Specificity = true negative number / (true negative number + false positive number) × 100%

Diagnostic accuracy = (true positive number + true negative number) / total number × 100%

2.6 BRAF mutation analysis

Tumor targets were manually microdissected from 4-μm unstained histologic sections. DNA was extracted from paraffin sections, using the DNeasy tissue kit (Qiagen, Valencia, CA), according to the manufacturer's instructions. Real-time polymerase chain reaction (PCR) was performed using allele specific primers designed to selectively amplify the wild-type (T1799) and mutant (A1799) BRAF alleles. After amplification, samples were subjected to a temperature ramp from 60 °C to 99 °C, increasing 1 °C at each step. For wild-type samples, single peaks were observed at 80 °C, whereas samples containing mutant alleles produced single peaks at 85 °C.

2.7 Statistics analysis

For analysis of clinicopathological factors, the enumeration data analysis between PEA group and MCRC group was carried out using the χ^2 -test and Fisher's exact test on IBM SPSS statistics 23 (IBM, New York, USA). *P*-values less than 0.05 were defined as significant (**P* < 0.05, ***P* < 0.01).

3. Results

3.1 SATB2 is highly Tissue-Specific and has Prognostic Value

We got the expression level of SATB2 in 27 different tissues from an article[12] with biological project number PRJEB4337 by pubmed, thereby obtaining Fig. 1. It can be seen that SATB2 expression was tissue-specific. It was highly expressed in intestinal tissues and brain tissues, and its expression level in lung tissues was very low.

3.2 The Performance of Common Immunomarkers in Diagnosis

As stated in Sect. 2.2 of the article, we preliminary screened (lung tumor vs PEA and MCRC) the patients with lung tumor according to HE and common immunohistochemistry in the WHO[1]. We secondary screened (PEA vs MCRC) the cases obtained from the preliminary screening after carefully analyzing clinical history, lower abdomen CT, colonoscopy and 6 months follow-up. We final obtained 28 cases of PEA and 42 cases of MCRC (Fig. 2).

In the preliminary screening, SATB2, CK7, CK20, CDX2, villin, TTF-1 and NapsinA play a great role. In order to show the effect of these immunomarkers, we randomly selected 60 cases of lung adenocarcinoma and 60 cases of CRC, and combined the final PEA and MCRC to evaluate the positive rate of these immunomarkers (Table 1). It suggested that PEA and MCRC had much higher positive rate of villin than lung adenocarcinoma. There were some differences in the positive rates of TTF-1, NapsinA, CK20 and CDX2 between lung adenocarcinoma and PEA. Between lung adenocarcinoma and MCRC, the positive rates of CK7, CK20, CDX2, villin, TTF-1 and NapsinA were also very different. In addition, we compared the positive rate of immunomarkers between CRC and MCRC. There was almost no difference between the two.

In the secondary screening, we analyze the positive rate of each immunomarker in PEA and MCRC (Table 1). In the patients with PEA, the positive rates of SATB2, CK7, TTF-1, NapsinA, CK20, CDX2 and villin were 0%, 96.43%, 14.29%, 7.14%, 25%, 50%, and 85.71%, respectively. In the patients with MCRC, the positive rates of SATB2, CK7, TTF-1, NapsinA, CK20, CDX2 and villin were 92.86%, 16.67%, 4.76%, 2.38%, 90.48%, 85.71%, and 92.86%, respectively. The positive rate of CK7 in PEA was very high. CK20, CDX2 and villin, which were seldom expressed in lung adenocarcinoma, were also expressed in some PEA cases. While TTF-1 and NapsinA, which were often expressed in lung adenocarcinoma, were rarely expressed in PEA. As for MCRC, many cases expressed intestinal cancer immunomarkers (CK20, CDX2 and villin), while few cases expressed lung adenocarcinoma immunomarkers (TTF-1, NapsinA). And the expression rate of CK7 in MCRC was very low. In contrast, the positive rate of SATB2 in PEA and MCRC is very different.

3.3 Differences between PEA and MCRC in Clinical Characteristics

We divide patients into PEA group and MCRC group. We performed the chi-square test to detect whether there were differences in age, gender, tumor size, tumor number, pleura invasion, bronchus invasion, lymph node metastasis, vessel invasion and nerve invasion between PEA and MCRC. As shown in Table 2, PEA and MCRC had differences in the number of tumor nodules. The tumors in the PEA group (single/total, 26/28) were mainly single, and the MCRC group (multifocal, 16/42) is almost equal on a single tumor and multiple tumors ($P = 0.004$).

3.4 SATB2 has the highest diagnostic value

The expression rates of SATB2 in PEA and MCRC were 0.00% (0/28) and 92.86% (39/42), respectively. For MCRC, the expression rate of common lung adenocarcinoma immunomarkers (CK7, TTF-1, and NapsinA) was all relatively low, and the expression rate of common intestinal cancer immunomarkers (CK20, CDX2, and villin) was all relatively high. However, among these immunomarkers, the positive and negative of some immunomarkers were equally distributed in PEA. In order to facilitate statistics and comparison, we took MCRC as the protagonist and calculated the sensitivity, specificity and diagnostic accuracy of various immunomarkers for distinguishing MCRC from PEA. The sensitivity of SATB2-, CK7+, TTF-1+, NapsinA+, CK20-, CDX2- and villin- for distinguishing MCRC from PEA were 92.86%, 83.33%, 95.24%, 97.62%, 90.48%, 85.71%, 92.86%, respectively. The specificity of SATB2-, CK7+, TTF-1+, NapsinA+, CK20-, CDX2- and villin- for distinguishing MCRC from PEA were 100.00%, 96.43%, 14.29%, 7.14%, 75%, 50%, 16.67%, respectively. The diagnostic accuracy was 95.71%, 88.57%, 62.86%, 61.43%, 84.29%, 71.43%, 61.43%, respectively (Table 3). As a result, the sensitivity, specificity and diagnostic accuracy of SATB2 were relatively high. Its diagnostic accuracy was the best, which is much higher than common lung adenocarcinoma immunomarkers (CK7, TTF-1, NapsinA) and intestinal cancer immunomarkers (CK20, CDX2 and villin). We can view that SATB2 is the best immunomarker for identifying PEA and MCRC. The diagnostic accuracy of CK7 was 88.57%, which was slightly inferior to SATB2, and the results of CK7 could be used as a reference for SATB2 differential diagnosis. The sensitivity of TTF-1 and Napsin A was 95.24% and 97.62%, respectively. They were also quite excellent and could be used as a reference for SATB2 differential diagnosis.

3.5 Mutation analysis results

In our results, there were 3 cases of MCRC specimens that showed negative for SATB2 staining. Protein expression in CRC is often influenced by underlying molecular alterations such as KRAS and BRAF mutations, and DNA mismatch repair (MMR) protein deficiency[13–18]. In Ma's research[19], loss of SATB2 expression was more commonly seen in CRC with MMR protein deficiency and BRAF mutation. However, in our study, there were no BRAF mutations and MMR protein deficiency in the 3 cases of MCRC specimens with negative SATB2 (figure omitted). This may be due to the poor differentiation of their cancer cells, so the SATB2 staining of the cancer cells can't show positive.

4. Discussion

PEA is characterized by the features of growth architectures of moderate-to-well differentiated glands, sometimes with a cribriform pattern formed, lined by tall columnar tumor cells with nuclear pseudostratification, dirty necrosis in the lumen and occasionally, prominent nuclear debris[1]. However, some of these morphologic features such as the tall columnar cells with brush-border and eosinophilic cytoplasm arranged in garland-like patterns can also be seen in MCRC. In our research, the expression rate of CK7 in PEA was very high; CK20, CDX2 and villin, which were not often expressed in lung adenocarcinoma, were also expressed in some PEA cases; while TTF-1 and NapsinA, which were often expressed in lung adenocarcinoma, was rarely expressed in PEA. As for MCRC, many cases expressed intestinal cancer immunomarkers (CK20, CDX2 and villin) and few cases expressed lung

adenocarcinoma immunomarkers (TTF-1, NapsinA). And the expression rate of CK7 in MCRC was very low. These immunomarkers have limited value in the differential diagnosis of PEA and MCRC. These have caused difficulties in diagnosis, but the treatment strategies and prognosis outcomes differ considerably for patients with the two malignancies[20]. Therefore, there is an urgent need to develop new markers to differentiate PEA and MCRC.

SATB2 is a nuclear matrix-associated transcription factor and a reliable marker of intestinal differentiation[11]. It serves as an important immunomarker to distinguish primary CRC from metastatic tumors[20]. SATB2 is expressed in 86%-93% of primary CRC and 81%-94% of MCRC[11], and the frequency of SATB2 expression in reported conventional lung adenocarcinoma is about 10%[7]. Here, we used bioinformatics to analyze the expression of SATB2 in the lung and colorectum. SATB2 is highly expressed in the colorectum and hardly expressed in the lung. The tissue-specific of SATB2 illustrates a theoretical basis of our research.

Although several studies on SATB2 expression in PEA have been published, our conclusions have once again updated our understanding of the differential value of SATB2. We expanded the number of PEA and MCRC patients included in the study. Previous studies have shown that SATB2 could distinguish between PEA and MCRC[21–23], but the identification value is limited, and we even need to combine it with other immunomarkers in order to achieve a clear identification effect. In Mark's research[23], they showed that the differential diagnosis value of SATB2 in pulmonary and colorectal adenocarcinomas. SATB2 was positive in 80 (8%) non-small cell lung cancers (NSCLC), among various types of NSCLC, the positive rate of SATB2 is the highest in pleomorphic carcinomas. And 78 (98%) CRC were SATB2-positive. In my opinion, PEA is a rare histological subtype of lung adenocarcinoma. There will be some differences in the immunohistochemical performance of PEA and lung adenocarcinoma. However, due to changes in the tumor microenvironment, the immunohistochemical performance of MCRC and CRC also showed differences. This article can only provide us with limited reference value. In Jun's research[21], the case 4 was a PEA sample, but its SATB2 immunohistochemical staining was positive. It is worth noting that the mutation analysis of this case showed that its exon 2 of KRAS had a G12V mutation. In Bian's research[22], 2 of 13 PEA specimens showed that SATB2 immunohistochemical staining was positive. But they found that the SATB2 staining was weakly positive in the two cases.

Figure 3 is a diagram of our research ideas, through this research, we found that SATB2 can largely identify the PEA and MCRC. The expression rates of SATB2 in PEA and MCRC were 0.00% and 92.86%, respectively. There were 3 cases of MCRC specimens that showed negative SATB2 staining, and these three cases were poorly differentiated and atypical intestinal adenocarcinoma. This may due to the poor differentiation of their cancer cells, leading to the failure of SATB2 staining. In Ma's research[19], loss of SATB2 expression is more commonly seen in CRC with MMR protein deficiency and BRAF mutation. But, in our study, there were no BRAF mutations and MMR protein deficiency in the 3 cases of MCRC specimens with negative SATB2.

The sensitivity, specificity and diagnostic accuracy of SATB2 were respectively 92.86%, 100% and 95.71%, which is much higher than common lung adenocarcinoma immunomarkers (CK7, TTF-1, Napsin A) and intestinal cancer immunomarkers (CK20, CDX2 and villin). We can view that SATB2 is the best immunomarker for identifying PEA and MCRC. The diagnostic value of CK7 is slightly inferior to SATB2, and the results of CK7 can be used as a reference for SATB2 differential diagnosis. The sensitivity of TTF-1 and Napsin A is 95.24% and 97.62%, respectively. They are also quite excellent and can be used as a reference for SATB2 differential diagnosis.

Abbreviations

Abbreviations	Full title
PEA	pulmonary enteric adenocarcinoma
MCRC	metastases of colorectal carcinoma
GI	gastrointestinal
CRC	colorectal carcinoma
SATB2	special AT-rich sequence binding-protein
PCR	polymerase chain reaction
MMR	mismatch repair
NSCLC	non-small cell lung cancers

Declarations

Ethics approval and consent to participate

After obtaining approval from the Ethical Committee of Renmin Hospital of Wuhan University (WDRY2019-K010), we launched this research project. All patients provided signed informed consent.

Consent for publication

No conflict of interest exists in the submission of this manuscript, and manuscript is approved by all authors for publication. I would like to declare on behalf of my co-authors that the work described was original research that has not been published previously.

Availability of data and materials

We collect patient information from 2015 to 2020 in the hospital's case database, and use the preserved wax blocks to make sections for follow-up research.

Competing interests

All authors declare no conflict of interest.

Funding

This work was supported by grants from Science and Technology Planning Project of Wuhan (Grant No. 2017060201010172) and Guidance Foundation of Renmin Hospital of Wuhan University (Grant No. RMYD2018M27).

Authors' contributions

All Authors' Acknowledgment: that all authors have contributed significantly, and that all authors are in agreement with the content of the manuscript.

Acknowledgments

We thank all the participants in this article for their efforts in this article. We are grateful to all the pathologists and technicians of Renmin Hospital of Wuhan University for their support and assistance. We are also thankful to the reviewers for critically assessing the manuscript.

Disclose

The abstract of this paper was presented at the USCAP 2020 Conference name "Differential Diagnostic Values of SATB2 in Primary Pulmonary Enteric Adenocarcinoma and Pulmonary Metastases of Colorectal Carcinoma" as a poster presentation with interim findings. The poster's abstract was published in "Poster Abstracts" in pathology Journal name "Mondern Pathology".

References

- 1 Travis WD, Brambilla E, Nicholson AG, Yatabe Y, Austin J, Beasley MB, Chirieac LR, Dacic S, Duhig E, Flieder DB, Geisinger K, Hirsch FR, Ishikawa Y, Kerr KM, Noguchi M, Pelosi G, Powell CA, Tsao MS, Wistuba I: The 2015 world health organization classification of lung tumors: Impact of genetic, clinical and radiologic advances since the 2004 classification. J THORAC ONCOL 2015;10:1243-1260.
- 2 Tot T: Cytokeratins 20 and 7 as biomarkers: Usefulness in discriminating primary from metastatic adenocarcinoma. EUR J CANCER 2002;38:758-763.
- 3 Moll R, Schiller DL, Franke WW: Identification of protein IT of the intestinal cytoskeleton as a novel type I cytokeratin with unusual properties and expression patterns. J CELL BIOL 1990;111:567-580.
- 4 Barbareschi M, Murer B, Colby TV, Chilosi M, Macri E, Loda M, Doglioni C: CDX-2 homeobox gene expression is a reliable marker of colorectal adenocarcinoma metastases to the lungs. AM J SURG PATHOL 2003;27:141-149.

- 5 Wang CX, Liu B, Wang YF, Zhang RS, Yu B, Lu ZF, Shi QL, Zhou XJ: Pulmonary enteric adenocarcinoma: A study of the clinicopathologic and molecular status of nine cases. *Int J Clin Exp Pathol* 2014;7:1266-1274.
- 6 Nottegar A, Tabbò F, Luchini C, Brunelli M, Bria E, Veronese N, Santo A, Cingarlini S, Gilioli E, Ogliosi C: Pulmonary adenocarcinoma with enteric differentiation: Immunohistochemistry and molecular morphology. *APPL IMMUNOHISTO M M* 2018
- 7 Dragomir A, de Wit M, Johansson C, Uhlen M, Ponten 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;141:630-638.
- 8 Hatanaka K, Tsuta K, Watanabe K, Sugino K, Uekusa T: Primary pulmonary adenocarcinoma with enteric differentiation resembling metastatic colorectal carcinoma: A report of the second case negative for cytokeratin 7. *PATHOL RES PRACT* 2011;207:188-191.
- 9 Sauter ER, Bolton JS, Willis GW, et al. Improved survival after pulmonary resection of metastatic colorectal carcinoma[J]. *J SURG ONCOL*, 1990, 3(43): 135-138.
- 10 Olga B, Sergey A, Sergey L, Peter G, Victor T: Novel transcription factor Satb2 interacts with matrix attachment region DNA elements in a tissue-specific manner and demonstrates cell-type-dependent expression in the developing mouse CNS. *EUR J NEUROSCI* 2015;21:658-668.
- 11 Magnusson K, de Wit M, Brennan DJ, Johnson LB, McGee SF, Lundberg E, Naicker K, Klinger R, Kampf C, Asplund A, Wester K, Gry M, Bjartell A, Gallagher WM, Rexhepaj E, Kilpinen S, Kallioniemi OP, Belt E, Goos J, Meijer G, Birgisson H, Glimelius B, Borrebaeck CA, Navani S, Uhlen M, O'Connor DP, Jirstrom K, Ponten F: SATB2 in combination with cytokeratin 20 identifies over 95% of all colorectal carcinomas. *AM J SURG PATHOL* 2011;35:937-948.
- 12 Fagerberg L, Hallstrom BM, Oksvold P, Kampf C, Djureinovic D, Odeberg J, Habuka M, Tahmasebpour S, Danielsson A, Edlund K, Asplund A, Sjostedt E, Lundberg E, Szigartyo CA, Skogs M, Takanen JO, Berling H, Tegel H, Mulder J, Nilsson P, Schwenk JM, Lindskog C, Danielsson F, Mardinoglu A, Sivertsson A, von Feilitzen K, Forsberg M, Zwahlen M, Olsson I, Navani S, Huss M, Nielsen J, Ponten F, Uhlen M: Analysis of the human tissue-specific expression by genome-wide integration of transcriptomics and antibody-based proteomics. *MOL CELL PROTEOMICS* 2014;13:397-406.
- 13 Kim JH, Rhee YY, Bae JM, Cho NY, Kang GH: Loss of CDX2/CK20 expression is associated with poorly differentiated carcinoma, the CpG island methylator phenotype, and adverse prognosis in microsatellite-unstable colorectal cancer. *AM J SURG PATHOL* 2013;37:1532-1541.
- 14 Lugli A, Tzankov A, Zlobec I, Terracciano LM: Differential diagnostic and functional role of the multi-marker phenotype CDX2/CK20/CK7 in colorectal cancer stratified by mismatch repair status. *Mod Pathol* 2008;21:1403-1412.

- 15 Zlobec I, Bihl M, Foerster A, Ruffe A, Lugli A: Comprehensive analysis of CpG island methylator phenotype (CIMP)-high, -low, and -negative colorectal cancers based on protein marker expression and molecular features. *J PATHOL* 2011;225:336-343.
- 16 McGregor DK, Wu TT, Rashid A, Luthra R, Hamilton SR: Reduced expression of cytokeratin 20 in colorectal carcinomas with high levels of microsatellite instability. *AM J SURG PATHOL* 2004;28:712-718.
- 17 Gurzu S, Jung I: Aberrant pattern of the cytokeratin 7/cytokeratin 20 immunophenotype in colorectal adenocarcinomas with BRAF mutations. *PATHOL RES PRACT* 2012;208:163-166.
- 18 Gurzu S, Szentirmay Z, Toth E, Bara T, Bara TJ, Jung I: Serrated pathway adenocarcinomas: Molecular and immunohistochemical insights into their recognition. *PLOS ONE* 2013;8:e57699.
- 19 Ma C, Olevian DC, Lowenthal BM, Jayachandran P, Kozak MM, Chang DT, Pai RK: Loss of SATB2 expression in colorectal carcinoma is associated with DNA mismatch repair protein deficiency and BRAF mutation. *AM J SURG PATHOL* 2018;42:1409-1417.
- 20 Osoegawa A, Kometani T, Fukuyama S, Hirai F, Seto T, Sugio K, Ichinose Y: Prognostic Factors for Survival after Resection of Pulmonary Metastases from Colorectal Carcinoma. *Ann Thorac Cardiovasc Surg* 2016;22:6-11.
- 21 Matsushima J, Yazawa T, Suzuki M, Takahashi Y, Ota S, Nakajima T, Yoshino I, Yokose T, Inoue T, Kawahara K, Nakatani Y: Clinicopathological, immunohistochemical, and mutational analyses of pulmonary enteric adenocarcinoma: Usefulness of SATB2 and beta-catenin immunostaining for differentiation from metastatic colorectal carcinoma. *HUM PATHOL* 2017;64:179-185.
- 22 Bian T, Zhao J, Feng J, Zhang Q, Qian L, Liu J, Jiang D, Liu Y, Zhang J: Combination of Cadherin-17 and SATB homeobox 2 serves as potential optimal makers for the differential diagnosis of pulmonary enteric adenocarcinoma and metastatic colorectal adenocarcinoma. *Oncotarget* 2017.
- 23 Kriegsmann M, Harms A, Longuespee R, Muley T, Winter H, Kriegsmann K, Kazdal D, Goeppert B, Pathil A, Warth A: Role of conventional immunomarkers, HNF4-alpha and SATB2, in the differential diagnosis of pulmonary and colorectal adenocarcinomas. *HISTOPATHOLOGY* 2018;72:997-1006.

Tables

Due to technical limitations, table 1, 2, 3 is only available as a download in the Supplemental Files section.

Figures

Figure 1

Analysis of the expression level of SATB2 in various tissues of the human body.

Figure 1

Analysis of the expression level of SATB2 in various tissues of the human body.

Figure 2

Staining effect of each immunomarker in PEA and MCRC.

Figure 2

Staining effect of each immunomarker in PEA and MCRC.

Figure 3

A diagram of research ideas

Figure 3

A diagram of research ideas

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Table1.xlsx](#)
- [Table1.xlsx](#)
- [Table2.xlsx](#)
- [Table2.xlsx](#)
- [Table3.xlsx](#)
- [Table3.xlsx](#)