Loss of SATB2 expression correlates with cytokeratin 7 and PD-L1 tumor cell positivity and aggressive behavior in colorectal cancer

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Abstract

Colorectal carcinoma (CRC) represents a health issue causing significant morbidity and mortality worldwide. Further biomarkers are needed to allow patient risk stratification in terms of prognosis. In this study, we aimed to clarify prognostic significance of colonic-specific transcription factor special AT-rich sequence-binding protein 2 (SATB2), cytoskeletal protein cytokeratin 7 (CK7), and immune checkpoint molecule programmed death ligand 1 (PD-L1) analyzing a cohort of 285 patients with surgically treated CRC, analyzing quantitative associations of all three markers and several traditional prognosticators (tumor stage, histological grade, variant morphology, laterality, mismatch-repair/MMR status). The results of the study include negative significant prognostic impact of loss of SATB2 expression in overall survival (OS) and cancer specific survival (CSS), significantly shortened 5 years OS and CSS and 10 years CSS in patients with CRC expressing CK7, and borderline insignificantly shortened OS in patients with PD-L1 + CRC. PD-L1 showed significant detrimental impact in case of stronger expression. Loss of SATB2 was associated with CK7 expression, advanced tumor stage, high grade, right-sided localization, and borderline insignificantly with PD-L1 expression. CK7 expression was associated with high grade. Both loss of SATB2 and CK7 expression were significant negative prognostic predictors in the multivariate analysis adjusting on associated parameters and patient age. In summary, loss of SATB2 expression and gain of CK7 and PD-L1 expression characterize aggressive phenotype of CRC.

Introduction

In 2020, colorectal carcinoma (CRC) represented with 1.93 million cases the third most frequent human malignancy and with 935 000 deaths the second most important cancer-related cause of death worldwide [1] and comprises about 10% of human malignant tumors and cancer-related deaths [2]. Localized tumors and subset of tumors with lymph node metastases may be curatively treated by surgical resection, whereas a proportion of these patients and cases with distant metastases may benefit from (neo)adjuvant treatment. The identification of further biomarkers is needed to identify high-risk tumors and to stratify the patients in terms of prognosis.

Special AT-rich sequence-binding protein 2 (SATB2) is an evolutionary conserved protein binding to matrix attachment regions activating gene transcription in a matrix attachment region-dependent manner, described in 2003 [3, 4]. Matrix attachment regions are regulatory DNA sequences that are important for higher-order chromatin organization, and extension of chromatin modifications. Two homologous proteins SATB1 and SATB2 bind to these DNA sequences with various regulatory functions in gene expression. In mice, SATB2 transcripts have been identified in pre-B cells, brain, kidney, thymus, and testis [4]. In humans, SATB2 is constitutively expressed and it has developmental roles in craniofacial, neural, and osteoblastic differentiation [3]. Its haploinsufficiency caused by deletion of several genes has been labeled to induce cleft palate and craniofacial malformations [5]. It has been shown that SATB2 is constitutively expressed in colonic mucosa - a recent murine study showed loss of colonic stem cell identity in case of SATB2 loss; and inversely, gain of SATB2 expression in small bowel stem cells lead to conversion to colonic phenotype [6]. In human pathology, SATB2-immunohistochemistry gained importance in the last two decades as a marker of osteosarcoma [7, 8]. SATB2 is constitutively expressed in the physiological colorectal mucosa and in a majority of colorectal adenocarcinomas [9–14], and it is widely used in routine as an immunohistochemical marker of colorectal origin to differentiate it from other adenocarcinoma primaries. Several previous studies described an association of diminished SATB2 expression with poor CRC prognosis [15–18].

Cytokeratins (CK7) are cytoskeletal structural proteins present in epithelia and in epithelial tumors. CRC is mostly expressing CK20 like normal colonic mucosa. CK7 occurs in various glands (breast, skin adnexa, salivary, pancreatobiliary ducts) and in many types of adenocarcinoma (breast, lung, pancreatobiliary, salivary) but also in a minority of CRC although CK20-CK7 + expression profile is often thought to indicate non-colorectal origin. The rate of positive CRCs varies between 0–22% in published studies [19–35]. According to several analyzes including our recent study, CK7-expression in CRC was associated with aggressive tumor properties in terms of more advanced stage [23, 26, 36], and shorter survival [27, 32, 34, 35].

Programmed death ligand 1 (PD-L1) is a cell surface molecule expressed in various immune and tumor cell types. PD-L1 expression allows tumor cells to escape antitumor cytotoxic immunity; and it has been described to worsen prognosis in several

gastrointestinal malignancies including gastric cancer [36–39], esophageal cancer [40, 41], and particular subtypes of pancreatic cancer [42, 43]. Despite only a small subset of CRC express PD-L1, there is evidence documenting its negative prognostic impact [44–56].

To identify a particularly aggressive CRC phenotype, we analyzed a similar cohort from our previous study [57] with nearly 10 years follow-up focusing on eventual association between SATB2, CK7, and PD-L1 expression, correlation with traditional prognostic parameters such as stage, grade, anatomical site, mismatch-repair (MMR) status; and its eventual prognostic implications. As a secondary aim, we analyzed the prognostic impact of PD-L1 expression according to its tumor cell expression rate.

Material And Methods

A number of 285 patients with surgically resected histopathologically verified adenocarcinoma of the colon and rectum from years 2010–2013 with known follow-up and formalin-fixed paraffin-embedded (FFPE) resection specimen tumor tissue available were found in the medical records of the pathology department. Cases of all stages were included, without selection according to neoadjuvant/adjuvant therapy. No patient received immune-checkpoint inhibitor treatment. Among these cases, there were conventional adenocarcinoma, mucinous adenocarcinoma and signet-ring adenocarcinoma. Grade and stage of the tumor was recorded based on medical records. Stage I-IV was assigned in accordance with TNM Classification [58] and Union for International Cancer Control (UICC). A tissue microarray (TMA) technique was used to make paraffin blocks for immunohistochemical slides using manual tissue arrayer TMA Master 3D Histech. From each paraffin block containing invasive adenocarcinoma tissue, two cylindrical samples measuring 2mm were taken from random spots of the tumor tissue. All samples were collected in a recipient paraffin TMA block. Each recipient block contained 20 samples from 10 cases. For immunohistochemistry, 4 µm-thick tissue sections were stained in a Ventana BenchMark ULTRA autostainer (Ventana Medical Systems, Tucson, Arizona). Monoclonal antibodies against SATB2 (CellMarque, EP281, 1:200), CK7 (clone OV-TL, BioSB, 1:500), PD-L1 (clone 22C3 pharmDx, Agilent, diagnostic kit), MutS homolog 2 (MSH2, clone G219-1129, Roche, ready to use), postmeiotic segregation 2 (PMS2, clone A16-4, Roche, ready to use), MutS homolog 6 (MSH6, clone 44, Roche, ready to use), MutL homolog 1 (MLH1, clone M1, Roche, ready to use) were used. The positive reactions were visualized by the Ultraview Detection System (Ventana Medical Systems), counterstaining the slides with hematoxylin. Stained slides were dehydrated and covered in a xylene-based mounting medium. All immunohistochemical examinations were assessed using a microscope by two experienced routine pathologists (JH and RM). In the case of SATB2, only nuclear staining was regarded as positive in both weak and strong staining; the percentage of positive tumor cells was recorded. The cohort was binarized according to an optimal cutpoint calculated using optimization of the log-rank test resulting in 40% of SATB2-positive cells as a cutoff value. Function surv_cutpoint (survminer) determines the optimal cutpoint using the maximally selected rank statistics from the `max` R package (see below).

For CK7, the percentage of positive tumor cells was recorded whereas staining in > = 10% of tumor cells was considered a positive sample. PD-L1 was considered positive only in case of membranous staining. The percentage of positive neoplastic cells (tumor proportion score - TPS) was recorded following standardized recommendations. Concerning MMR status, tumors with any apparent nuclear staining with MSH2, MSH6, PMS2, and MLH1 were considered MMR-proficient. Tumors with obvious loss of nuclear staining of anti-MMR antibodies with control positivity in stroma and lymphocytes were considered MMR-deficient. All microscopical analyses were performed without knowledge of clinical data and patient follow-up (Fig. 1).

Overall survival (OS) and cancer-specific survival (CSS) were calculated from the date of surgery to date of recorded death or to the last known follow-up date (censoring). In regard to CSS, the patients with non-CRC-related causes of death were censored at the date of death. Separate analysis for 10years and 5years follow-up were performed. For survival analysis, we performed a univariate Kaplan-Meier analysis with the log-rank test and confidence intervals calculated using the log-log method. To assess eventual associations between examined variables, logistic regression (Pearson's chi-squared test) was calculated.

Furthermore, we performed a separate 5 years OS and CSS analysis on three subgroups divided as follows: PD-L1 negative and CRCs expressing PD-L1 in 1% in tumor cells (negative and weak expressors), moderate expressors (2–49%), and strong

expressors (50–100%). As an additional step, we binarized the cohort instead of arbitrary 1% cutoff using optimal cutpoint using optimization of the log-rank test as mentioned above resulting in 2% of PD-L1 positive tumor cells as a cutoff value; and the separate 5 years OS and CSS analysis according to this optimal cutpoint.

The survival analysis and logistic regression were performed separately for binarized cohort as follows: SATB2+ (> 40%) versus SATB2- (< = 40%), CK7+ (> = 10%) versus CK7- (< 10%), PD-L1+ (> = 1%) versus PD-L1- (< 1%), low grade (grade 1 + 2) versus high grade (grade 3), conventional adenocarcinoma versus adenocarcinoma with variant morphology (mucinous + signet ring carcinoma), MMR-proficient versus MMR-deficient, right sided tumors (cecum, ascendens, hepatic flexure, transversum) versus left sided tumors (lienal flexure, descendens, sigmoid, rectum), localized tumors (UICC stages 1 + 2) versus advanced tumors (UICC stages 3 + 4).

In the next step, multivariate Cox regression adjusting parameters with significant association from logistic regression and adjusting analyzed variables on the patient's age was performed.

P values < 0.05 were considered statistically significant. All analyses were performed in R version 4.0.3 (2020-10-10) [58]; survival analysis using package survival version 3.2–7 [59].

The publication was approved by University Hospital Královské Vinohrady ethical committee, approval number EK-R/04/012022. The University Hospital Královské Vinohrady waived the need for informed consent due to the retrospective nature of the study. The entire research has been performed in accordance with the Declaration of Helsinki.

Results

The cohort consisted of 161 male and 124 female patients, with mean age 68.55 years, median age 69 years, mode 70 years, range 30-94 years. All variables analyzed in the study are summarized in Supplementary table 1. Results of 10 years OS and CSS analysis are summarized in Table 1, 5 years OS and CSS in Table 2. Kaplan-Meier curves showing prognostic impact of SATB2 negativity, CK7 positivity and PD-L1 positivity are shown in Figure 2.

The patients with SATB2- tumors (n=54) displayed significantly shorter both OS (restricted mean/rmean = 4.921 years versus 6.943 years, hazard ratio=0.511, p=0.00042) and CSS (rmean = 5.633 years versus 7.648 years, HR=0.452, p=0.00027) compared to those with SATB2+ tumors (n=231) in 10 years follow-up. Analyzing 5 years of follow-up, there was significantly shorter both OS (rmean = 3.157 versus 4.008 years, HR=0.46, p=0.00019) and CSS (rmean = 3.494 versus 4.171 years, HR=0.464, p=0.0012) in the patients with SATB2- tumors.

In the 10 years follow-up, CK7 expression showed detrimental prognostic impact with borderline insignificantly shorter OS (rmean 4.9 years versus 6.677 years, HR=1.695, p=0.077) and significantly shorter CSS (rmean = 5.468 years versus 7.412, HR=1.999, p=0.035) comparing CK7+ (n=19) with CK7- (n=266) cases. In the 5 years follow-up, there was negative prognostic impact of CK7 expression with significantly shorter both OS (rmean = 3.124 versus 3.898, HR=2.128, p=0.012) and CSS (rmean = 3.378 versus 4.096 years, HR=2.28, p=0.012).

In 10 years follow up, PD-L1 expression showed insignificantly shorter OS (rmean = 5.527 years versus 6.670 years, HR=1.46, p=0.15) and no significant difference in CSS (rmean = 7.29 years versus 7.329 years, HR=0.984, p=0.97) between PD-L1+ (n=28) and PD-L1- (n=257) CRCs. In 5 years follow up, PD-L1+ tumors displayed insignificantly shorter OS (rmean = 3.261 versus 3.91 years, HR=1.522, p=0.156) and no differences in CSS (rmean = 3.948 versus 4.063, HR-1.016, p-0.97) compared to PD-L1- cases.

PD-L1 was further analyzed categorizing the patients according to percentage of tumor cell PD-L1 expression with following results (Table 3, Figure 3ab): negative cases (0%) and weak expressors (1%) comprising 261 cases displayed significantly longer OS compared to both moderate (2-49%, n=20) and strong (50-100%, n=4) expressors (rmean = 3.927 versus 3.136 versus 2.148 years, respectively, p=0.021). Analyzing CSS with PD-L1 percentage categorization, there were no significant differences (rmean = 4.077 versus 3.747 versus 3.838, p=0.78).

Prognostic impact of PD-L1 status was additionally calculated according to optimal cutpoint at 2% of positive tumor cells (Table 3, Figure 3cd). In this analysis, patients with CRC expriming PD-L1 in >2% of tumor cells (n=19) showed significantly shorter OS (rmean = 2.675 versus 3.930 years, p=0.0018) compared to those with PD-L1 negative tumors or weak expressors (n=266). The patients with CRC expriming PD-L1 in >2% of tumor cells displayed also shorter CSS compared to PD-L1 negative cases and weak expressors (rmean = 3.583 versus 4.078 years, p=0.3) but the difference lacked statistical significance (Table 3).

Among traditional prognostic variables, there was a significant negative prognostic impact of advanced UICC stage (p<0.0001), and grade 3 (p=0.013) on 10 years of OS. Concerning 10 years of CSS, there was a significant worsening effect of MMR-proficient status (p=0.0091), advanced UICC stage (p<0.0001), grade 3 (p=0.021), and borderline insignificant effect of right-sided tumor site(p=0.064) - see Table 1. In 5 years survival analysis, OS was significantly shorter in case of advanced UICC stage (p<0.0001), grade 3 (p=0.0031), right sided tumors (p=0.012), and carcinomas with mucinous or signet ring cell morphology (p=0.046), whereas CSS was worse in tumors with advanced UICC stage (p<0.0001), grade 3 (p=0.012), right sided tumors (p=0.012), and MMR-proficient CRCs (p=0.028) - see Table 2.

Table 1

10 years follow up - survival analysis - univariate Kaplan-Meier analysis with the log-rank test, Cox regression

	n	%	All deaths	Restricted mean OS (years)	OS Hazard ratio	OS p value	CRC related deaths	Restricted mean CSS (years)	CSS Hazard ratio	CSS p value
SATB2 >= 40%	54	18.9	36	4.921	0.511	0.00042	28	5.633	0.452	0.00027
SATB2 >40%	231	81.1	104	6.943			70	7.648		
CK7 >=10%	19	6.7	12	5.163	1.585	0.127	10	5.468	1.999	0.035
CK7 negative	266	93.3	135	6.751			88	7.412		
PD-L1 >= 1%	28	9.8	16	5.527	1.46	0.15	8	7.329	0.984	0.97
PD-L1 negative	257	90.2	124	6.670			90	7.290		
MMR-deficient	25	8.8	9	7.81	1.652	0.145	2	9.237	5.285	0.0091
MMR-proficient	260	91.2	131	6.44			96	7.107		
UICC I+II	143	50.2	51	7.769	2.371	<0.0001	24	8.757	4.081	<0.0001
UICC III+IV	142	49.8	89	5.34			74	5.83		
Adenocarcinoma NOS	269	94.4	130	6.654	0.566	0.084	6	6.287	0.685	0.37
Mucinous+signet ring carcinoma	16	5.6	10	4.967			92	7.338		
Grade 1+2	205	73.0	93	6.954	1.569	0.013	63	7.649	1.65	0.021
Grade 3	76	27.0	44	5.570			32	6.412		
Right sided CRC	112	39.3	60	5.924	0.745	0.084	44	6.641	0.688	0.064
Left sided CRC	173	60.7	80	6.973			54	7.705		

Table 2

5 years follow up - survival analysis - univariate Kaplan-Meier analysis with the log-rank test, Cox regression

	n	%	All deaths	Restricted mean OS (years)	OS Hazard ratio	OS p value	CRC related deaths	Restricted mean CSS (years)	CSS Hazard ratio	CSS p value
SATB2 >= 40%	54	18.9	31	3.157	0.46	0.00019	24	3.493	0.464	0.0012
SATB2 >40%	231	81.1	76	4.008			59	4.171		
CK7 >=10%	19	6.7	12	3.124	2.128	0.012	10	3.378	2.28	0.012
CK7 negative	266	93.3	95	3.898			73	4.096		
PD-L1 >= 1%	28	9.8	13	3.261	1.522	0.156	7	3.948	1.016	0.968
PD-L1 negative	257	90.2	94	3.910			76	4.063		
MMR-deficient	25	8.8	5	4.317	2.162	0.085	2	4.672	4.255	0.028
MMR-proficient	260	91.2	102	3.801			81	3.992		
UICC I+II	143	50.2	33	4.383	2.879	<0.0001	19	4.624	4.289	<0.0001
UICC III+IV	142	49.8	74	3.306			64	3.477		
Adenocarcinoma NOS	269	94.4	98	3.894	0.505	0.046	77	4.071	0.592	0.21
Mucinous+signet ring carcinoma	16	5.6	9	3.051			6	3.620		
Grade 1+2	205	73.0	67	4.038	1.81	0.0031	52	4.206	1.776	0.012
Grade 3	76	27.0	38	3.348			29	3.647		
Right sided CRC	112	39.3	51	3.502	0.619	0.013	41	3.695	0.578	0.012
Left sided CRC	173	60.7	56	4.070			42	4.278		

Table 3

5 years follow up - survival analysis according to percentage of PD-L1 expression - univariate Kaplan-Meier analysis with the log-rank test, Cox regression

	n	%	All deaths	Restricted mean OS (years)	OS p value	CRC related deaths	Restricted mean OS (years)	CSS p value
PD-L1 50-100%	4	1.4%	3	3.927	0.021	1	3.838	0.78
PD-L1 2-49%	20	7.0%	10	3.136		6	3.747	
PD-L1 <= 1%	261	91.6%	94	2.148		76	4.077	
PD-L1 >= 2%	19	6.7%	12	2.675	0.0018	6	3.583	0.3
PD-L1 <2%	266	98.3%	95	3.930		77	4.078	

Logistic regression analysis (Pearson's chi-squared test) revealed following significant associations: SATB2- tumors proned more to be in advanced stage (35/107 seu 24.6/75.4% were SATB2-/SATB2+ in UICC stages I+II and 19/127 seu 13.3/86.7% were SATB2-/SATB2+ in UICC stages III+IV, Odds ratio=0.468, p=0.016), high-grade (20/56 seu 26.3/73.7% were

SATB2-/SATB2+ with grade 3 and 32/173 seu 15.6/84.4% were SATB2-/SATB2+ with grades 1+2, OR=0.518, p=0.042), with variant mucinous or signet-ring cell morphology (47/222 seu 17.5/82.5% were SATB2-/SATB2+ conventional adenocarcinomas and 7/9 seu 43.8/56.2% were SATB2-/SATB2+ mucinous or signet ring cell carcinomas, OR=3.674, p=0.014), right-sided (29/83 seu 25.9/74.1% were SATB2-/SATB2+ in right sided CRCs and 25/148 seu 14.5/85.5% were SATB2-/SATB2+ in left sided CRCs, OR=2.068, p=0.017), and cytokeratin 7 positive (13/6 seu 68.4/31.6% were SATB2-/SATB2+ CK7+ tumors and 41/225 seu 15.4/84.6% were SATB2-/SATB2+ CK7- tumors, OR=0.084, p<0.001, Figure 4). Furthermore, there was a borderline insignificant association of SATB2 negativity with PD-L1 expression in CRC (9/19 seu 32.1/67.9% were SATB2-/SATB2+ PD-L1+ tumors and 45/212 seu 17.5/82.5% were SATB2-/SATB2+ PD-L1- tumors, OR=0.448, p=0.066, Figure 5). A relationship between PD-L1 expression and MMR status was without significant difference (7/18 seu 28/72% were SATB2-/SATB2+ MMR deficient tumors and 47/213 seu 18.1/81.9% were SATB2-/SATB2+ MMR proficient tumors, OR=1.762, p=0.232).

CK7 positive tumors were more frequently high-grade (10/66 seu 13.2/86.8% were CK7+/CK7- with CRC grade 3 and 9/196 seu 4.4/95.6% were CK7+/CK7- with CRC grade 1+2, OR=3.3, p=0.013). No association of CK7 expression with UICC stage, morphology, laterality, PD-L1 status, and MMR status was found.

PD-L1+ CRCs were more frequently MMR-deficient compared to the PD-L1- tumors (6/19 seu 24/76% were PD-L1+ MMRdef/MMRprof and 22/238 seu 8.5/91.5% were PD-L1- MMRdef/MMRprof, OR=0.0293, p=0.018). No association of PD-L1 positivity with UICC stage, grade, morphology, laterality, and CK7 status was identified.

The significant association of CK7 expression and SATB2 negativity and the borderline insignificant association of PD-L1 expression with SATB2 negativity were described above.

In the next step, prognostic impact of SATB2 and CK7 on 5years CSS and the impact of PD-L1 status on 5years OS (since the PD-L1-impact on CSS was not significant) was analyzed in multivariate Cox regression adjusting these on associated variables. 5years survival was analyzed in multivariate analysis due to higher level of statistical significance compared to 10years survival.

In the multivariate analysis, SATB2 negativity remained a significant poor 5 years CSS predictor if adjusted on patient's age (HR=0.469, p=0.002), UICC stage (HR=0.557, p=0.17), histopathological grade (HR=0.497, p=0.005), mucinous and signet ring cell morphology (HR=0.481, p=0.003), laterality (HR=0.499, p=0.005), CK7 expression (HR=0.53, p=0.016), and PD-L1 status (HR=0.469, p=0.002). In multivariate Cox regression, CK7 expression remained an independent detrimental 5 years CSS predictor if adjusted on patient´s age (HR=2.365, p=0.011), histopathological grade (HR=2.066, p=0.036), and SATB2 negativity (HR=0.53, p=0.016), as mentioned above. PD-L1 positivity of all percentages remained a borderline insignificant predictor of shorter 5years OS if adjusted on MMR status (HR=1.452, p=0.215).

In summary, our study revealed significantly shorter OS and CSS in patients with SATB2- and CK7+ CRC independently from associated analyzed factors, and borderline insignificantly shorter OS in patients with PD-L1+ CRC. SATB2 negative tumors were significantly more frequently CK7+, right-sided and presented in the advanced UICC stage. Association of SATB2 loss with PD-L1 expression was borderline insignificant (p=0.066). PD-L1 expression is borderline insignificant negative OS predictor without significant predictive value of CSS. Strong and moderate PD-L1 expression (>2% of tumor cells) is a significantly detrimental OS predictor without impact on CSS.

Discussion

SATB2 is widely used by pathologists as a sensitive and specific marker of colorectal origin in adenocarcinomas. As expressed in the majority of CRCs, loss of SATB2 has been identified as a detrimental prognostic marker in several studies. Wang et al. described an association of SATB2-negativity with presence of both lymph nodal and distant metastases, advanced Dukes` stage, shorter overall and disease free survival in CRC [15]. Mezheyeuski et al. linked strong SATB2 expression to left-sided tumor localization, low-grade, non-mutated BRAF status, longer overall survival, and better responsiveness to chemotherapy [17]. Schmitt et al. recently compared negative prognostic roles of SATB2 loss and caudal type homeobox transcription factor 2 (CDX2) loss: the authors have shown negative prognostic relevance of SATB2 loss in both univariate and multivariate analyses

and it`s prognostic superiority compared to loss of CDX2 [18]. Eberhart et al. described SATB2 expression as an independent favorable prognostic marker in CRC and as a predictor of response to adjuvant and neoadjuvant chemotherapy [16]. In concern to SATB2 as the chemotherapy response predictor, the favorable value of its expression in CRC [16] sharply contrasts with its negative therapy-predictive value in different cancers, i.e. head and neck squamous carcinoma [61]. This contradiction illustrates a complex role of SATB2 in cancer biology as it has been primarily described as a transcription factor in craniofacial embryogenesis [62]. In CRC, SATB2 should be regarded as a differentiation marker and thus, as a beneficial feature of low-risk phenotype, since it may act as an oncogene in non-colonic cell populations. SATB1 is a member of the same protein family which has been described as a cancer promoter in breast tumors and has been linked to poor prognosis [63]. Inversely to SATB2, SATB1 has been linked to rectal cancer progression [64] but without survival analysis.

Eberhart et al. documented significant association of SATB2 expression and microsatellite stability [16]. Our data suggest a slight enrichment of MMR-deficient status in SATB2 negative tumors although the difference did not reach statistical significance. Similarly, loss of widely known colorectal differentiation markers CK20 and CDX2 has been described to be more frequent in MMR-deficient tumors [65]. This may be explained through genomic instability and high mutation load in MSI CRC which lead to the loss of intestinal markers.

Our data are in line with well established research leading to identification of SATB2 loss as a robust marker of poor prognosis in CRC as we found significantly shorter OS and CSS, inclination to advanced UICC stage, right-sided localization and high-grade, whereas the prognostic value of SATB2 loss was independent negative predictor in the multivariate analysis. As a novel finding, we identified a strong association of SATB2 loss with CK7 and borderline insignificant (p = 0.066) association with PD-L1 expression. Both CK7 and PD-L1 expression were negative prognosticators as well. We believe that SATB2 and CK7 immunohistochemistry allow us to identify particularly aggressive CRC phenotypes.

Concerning CK7, its negative prognostic role and association with detrimental traditional factors (particularly stage) has been documented in several studies including our dataset [26, 27, 32, 34, 35, 57] whereas some recent studies shared the methodology in terms of 10% positivity cut-off value to regard the tumor CK7 positive [34, 35, 57]. According to Fei et al. [35] and Kirchner et al. [66], CK7 expression may be regarded as a hallmark of retrodifferentiation or dedifferentiation related to re-acquirement of fetal phenotype which is linked to epithelial-mesenchymal transition giving the tumor a capability to metastasize. Kirchner et al. described lack of glandular differentiation and primitive duct-like morphology in gastric mucosa expressing CK7 [66]. All these facts characterize CK7 expression in CRC as a dedifferentiation marker linked to more aggressive behavior.

PD-L1 have been widely studied in various types of cancer, multiple immunohistochemistry assays have been approved as a complementary diagnostic for patients with non-small cell lung cancer (NSCLC), melanoma, urothelial bladder cancer, esophageal and gastric cancer [67, 68]. In these tumors, PD-L1 is regarded as a target molecule of immune-checkpoint therapy (i.e. nivolumab, pembrolizumab) with significant benefit in patients' survival. Nevertheless, despite wide use of PD-L1 expression shown by immunohistochemistry as a biomarker for PD-1/PD-L1 blockade in many types of cancer, there are growing issues regarding its true predictability [69]. In case of CRC, the US Food and Drug Administration approved nivolumab as a treatment modality in MMR-deficient/MSI-H metastatic CRC refractory to fluoropyrimidine, oxaliplatin, and irinotecan [70], whilst PD-L1 has not yet been approved as a immunotherapy predictive marker. Concerning the predictive value of tumor cell PD-L1 expression in CRC, there are inconsistent results in various studies including meta-analyses. There are at least thirteen studies describing its detrimental prognostic impact [44–56], three studies found its beneficial effect [71–73], and five studies did not find any significant impact of tumor cell PD-L1 expression on CRC survival [74–78]. Among recent meta-analyses, we have found two describing negative impact [79, 80] and one describing slightly no reliable prognostic value [81].

Our results of PD-L1 expression analysis in CRC are in line with ambiguous results of cited studies: we shown slight (borderline insignificant with p value = 0.15) negative impact on OS and no impact on CSS, but there was significantly shorter OS in cases moderately and strongly (> 2%) expressing PD-L1 on tumor cell membrane. Analyzing CSS, no significant results were obtained. Our CSS analysis failed most likely due to the low number of cases with moderate and strong PD-L1 expression (> = 2%) comprising only 24 probands.

The contradictory results of various studies may be explained by 1) overall paucity of CRCs expressing PD-L1 even in larger datasets usually occurring in < 10% of probands with difficulties to reach statistical significance; 2) different methods of PD-L1 expression assessment either in terms of methodology (tumor proportion score - TPS, combined positive score - CPS, immune cell score - IC), or in terms of different used antibodies (e.g. clones 22C3, SP263), or in terms of percentage threshold value to regard a tumor as positive. In our study, we used a standardized diagnostic kit (SP263) and TPS was assessed. The results of our study suggest minimal prognostic impact of weak PD-L1 expression in 1% of tumor cells. Our data clearly document that only membranous PD-L1 expression in a substantial subset (> = 2% and particularly > 50%) of malignant cells is linked to poor survival. From the biological point of view, this may be explained by PD-L1 on tumor cell surface switching-off the cytotoxic T-lymphocytes enabling the tumor cell to escape from antitumor immunity. In NSCLC, several studies documented variable immune-checkpoint inhibitors (pembrolizumab) therapeutic effect according to different tumor cell percentage expressing PD-L1 assessed by immunohistochemistry, using positivity cut-off values of ≥ 1% [82, 83] or ≥ 50% [84]. The results of our research indicate using either a higher cut-off value about 2–5% to regard a CRC as PD-L1 + for patient prognostic stratification, despite being limited by a small number of patients with CRC moderately (n = 20) and strongly (n = 4) expressing PD-L1.

As already mentioned, all the three markers examined in our study (SATB2 negativity, CK7 and stronger PD-L1 positivity) may serve to prognostic stratification of patients suffering from CRC in all stages. However, this “aggressive CRC phenotype” may be characterized by dedifferentiation: loss of differentiation marker, production of aberrant cytokeratin 7, and eventual high neoantigen production, i.e. PD-L1. The exact link between those three markers is hard to explain, and needs further clarification at molecular level - we hypothesize about loss of constitutive colon-specific protein (SATB2) and gain of aberrant non-colonic molecules CK7 and PD-L1, i.e. possibly via genomic instability or high mutation load - this need to be further explored. Our study is limited by overall paucity of CK7 + and PD-L1 + CRCs; eventual corroboration of these associations in larger patient cohorts may be of great interest.

Declarations

Author's contribution:

JH, AN, IT, HF and KJ collected the clinical and histopathological data. JH and RM performed the microscopical evaluation of immunohistochemistry. JH and RM designed the experiment and wrote the paper. PW performed the statistical analysis.

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Conflicts of interest:

There are no conflicts of interests.

Data availability:

All used research data are available in appendix (electronic only).

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Figures

Figure 1

histological slides showing colorectal carcinoma (CRC), hematoxylin-eosin (HE) stain and immunohistochemistry, magnification 20x: a - conventional adenocarcinoma with strong nuclear SATB2 positivity, CK7 negativity, and PD-L1 positivity in immune cells and negativity in tumor cells; b - conventional adenocarcinoma with SATB2 negativity (staining in ca 1% tumor cells), strong cytoplasmic CK7 expression in 100% tumor cells, and strong membranous PD-L1 expression in ca 60% tumor cells; c - conventional adenocarcinoma with SATB2 negativity, strong cytoplasmic CK7 positivity in 100% tumor cells, and PD-L1 negativity; d - signet ring cell carcinoma with intracellular and extracellular mucin production showing SATB2 negativity (weak staining in ca 20% tumor cells), CK7 negativity, and PD-L1 negativity.

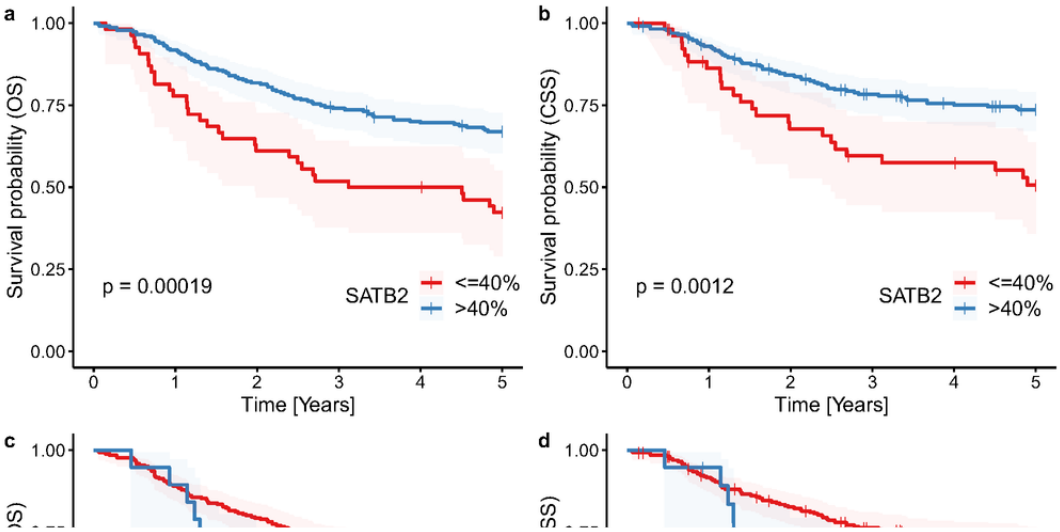

Figure 2

Kaplan Meier curves documenting significantly worse both 5 year overall survival (OS) and cancer specific survival (CSS) in patients with CRC with decreased or lost SATB2 expression according to an optimal cutpoint at 40% tumor cells (a, b); and in patients with CRC expressing CK7 in >=10% tumor cells (c, d); borderline insignificantly worse OS in patients with CRC expressing PD-L1 in >=1% tumor cells (e), and no significant impact of PD-L1 expression on CSS (f).

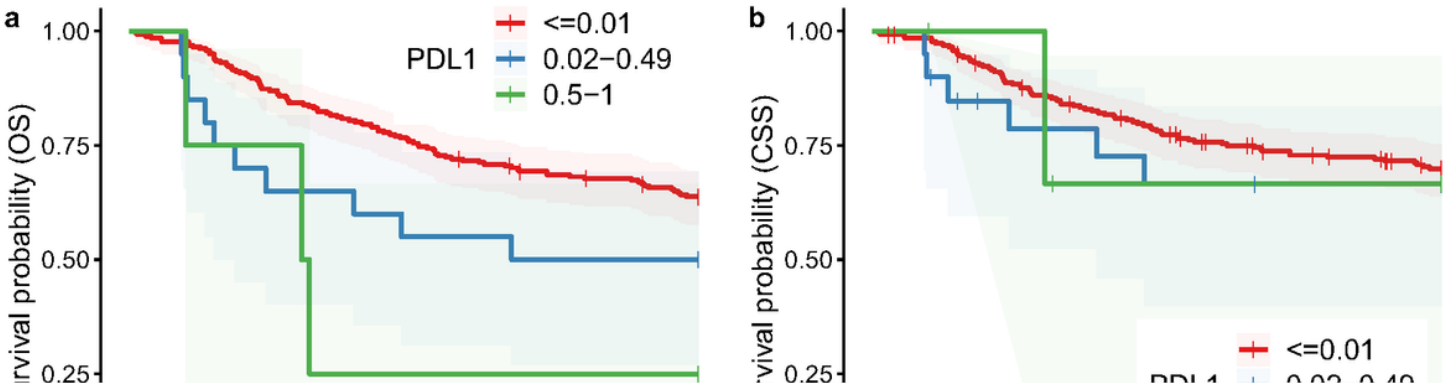

Figure 3

Kaplan Meier curves documenting significantly worse 5 year overall survival (OS) in patients with CRC moderately (2-49% tumors cells) and strongly (50-100% tumor cells) expressing PD-L1 (a) and with CRC showing both moderate and strong (2-100% tumor cells) PD-L1 expression according to optimal cutpoint (c); and no significant differences in cancer-specific survival (CSS, b, d).

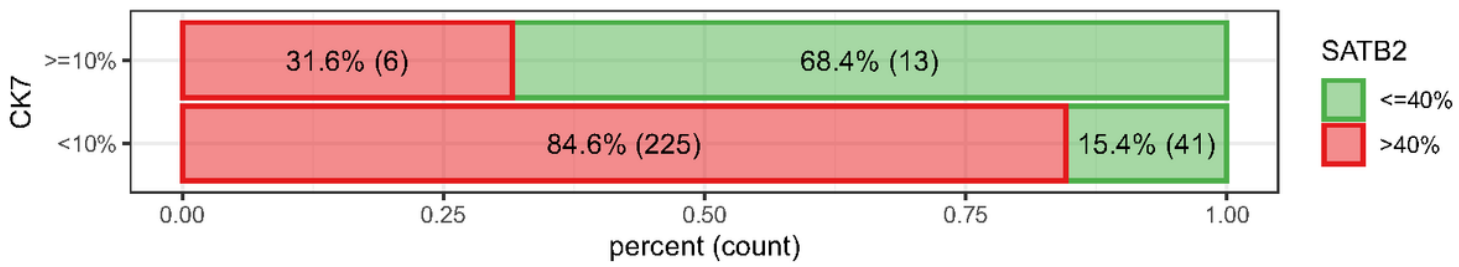

Figure 4

bar chart documenting significant enrichment of SATB2 negative CRCs with CK7 expression (logistic regression, p<0.001).

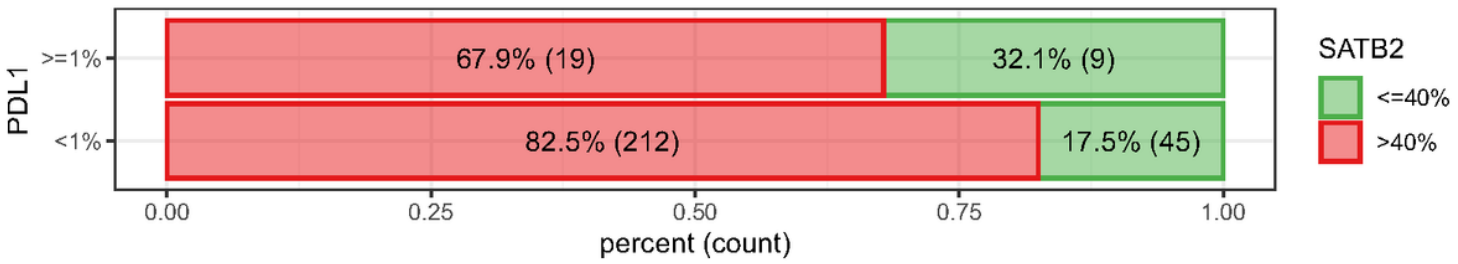

Figure 5

bar chart documenting borderline insignificant enrichment of SATB2 negative CRCs with PD-L1 expression in >=1% tumor cells (logistic regression, p=0.066).

Supplementary Files

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

- Supplementarytable1.xls