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Loss of SMARCB1 expression in colon carcinoma

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Abstract. SMARCB1 is a tumor suppressor gene, which is part of SWI/SNF complex involved in transcriptional regulation. Recently, loss of SMARCB1 expression has been reported in gastrointestinal carcinomas. Our purpose was to evaluate the incidence and prognostic value of SMARCB1 loss in colon carcinoma (CC).

Patients with stage III CC (n = 1695), and a second cohort of 23 patients with poorly differentiated CC were analyzed. Immunohistochemistry for SMARCB1 was performed on tissue microarrays, and cases with loss of expression were controlled on whole sections. Loss of SMARCB1 was compared with the clinico-pathological and molecular characteristics, and the prognostic value was evaluated.

Loss of SMARCB1 was identified in 12 of 1695 (0.7%) patients with stage III CC. Whole section controls showed a complete loss in only one of these cases, corresponding to a medullary carcinoma. SMARCB1 loss was not associated with histological grade, tumor size nor survival. In the cohort of poorly differentiated CC, we detected 2/23 (8.7%) cases with loss of SMARCB1; one was rhabdoid while the other had medullary and mucinous histology. These 2 cases were deficient for MisMatched Repair (dMMR) and mutated for *BRAF*.

SMARCB1 loss is rare in stage III CC, but appears more frequent in poorly differentiated CC.

Keywords: *SMARCB1*, colon carcinoma, *BRAF* V600E, mismatch repair deficiency

deficient for MisMatched Repair Immunohistochemistry		
frozen tissue and formalin-fixed		
paraffin-embedded tissue		
Microsatellite instability		
The Pan-European Trials in		
Alimentary traCt Cancer		
Progression-free survival		

SMARCB1SWI/SNF-related matrix-associated
actin-dependent regulator of
chromatin subfamily B member 1TMATissue microarray

5 1. Background

SMARCB1 (SWI/SNF-related matrix-associated 6 actin-dependent regulator of chromatin subfamily B 7 member 1) is a chromatin-remodeling gene and a tu-8 mor suppressor gene located on chromosome 22q11 [6, 9 8,18]. SMARCB1 encodes for the protein SMARCB1 10 which is part of the multimolecular SWI/SNF complex 11 involved in transcriptional regulation. The biallelic in-12 activation of SMARCB1 was first described in pediatric 13 rhabdoid tumors, and is the main oncogenic mech-14 anism. Then, loss of SMARCB1 protein expression 15 has been reported in a range of malignant neoplasms 16 including pediatric rhabdoid tumors (atypical tera-17 toid/rhabdoid tumors of the nervous system and malig-18 nant rhabdoid tumours) [5], epithelioid sarcomas [8,9] 19 and renal medullary carcinoma [7]. SMARCB1 mu-20 tation/inactivation has been reported to be correlated 21 with the loss of nuclear expression of SMARCB1 22 determined by immunohistochemistry (IHC) [8], and 23 IHC for SMARCB1 is already used by pathologists to 24 confirm the diagnosis of rhabdoid tumor. 25

Recently, SMARCB1 loss has been described in 26 series of gastrointestinal adenocarcinomas with rhab-27 doid morphology, including colon adenocarcinomas 28 (CC) [1,11,12,19]. Wang showed that the loss of 29 SMARCB1 in CC was rare (0.46%), and associ-30 ated with higher histological grade, larger tumor size, 31 lower survival, MSI and BRAF V600E status (p <32 0.001). However, the frequency and prognostic value 33 of SMARCB1 loss according to the stages of CC re-34 mains unclear. We therefore wanted to evaluate the in-35 cidence as well as the clinical pathological and molec-36 ular associations of SMARCB1 loss in a large, ho-37 mogenous and well characterized cohort of patients 38 with stage III CC, included in a prospective clinical 39 trial and all receiving FOLFOX adjuvant. 40

41 2. Methods

42 2.1. Patients

The stage III CC series corresponds to 2043 patients included in the PETACC8 study [13], who signed a specific consent for translational research and for whom FFPE samples were available. PETACC8 is an open, randomized, controlled, multi-center, multinational, phase 3 study in 18–75 years old patients, who underwent complete resection of stage III colon carcinoma (clinical trials # NCT00265811). Patients were randomized to receive adjuvant therapy: 6 months of FOLFOX 4 or FOLFOX and Cetuximab. The study was done in accordance with the Declaration of Helsinki (amended 2000) and the International Conference on Harmonization of Technical Requirements of Pharmaceuticals for Human Use (ICH) Note for Guidance on Good Clinical Practice and approved by the appropriate Ethics Committees.

The second series consists in 23 patients with poorly differentiated CC treated in Ambroise Paré hospital (Boulogne, France) or Laennec hospital (Nantes, France). In particular, the serie of Nantes consisted in 17 medullary carcinomas.

2.2. SMARCB1 immunohistochemistry

Immunohistochemistry for SMARCB1 was performed using a mouse monoclonal antibody (clone 25/BAF47, BD Bioscience). SMARCB1 staining was interpreted by two observers (JFE, SM) who were blinded to clinical, pathological and molecular data at the time of analysis. The loss of SMARCB1 expression was established when the nuclei of tumor cells were not stained and the adjacent stromal cells, inflammatory and/or endothelial component as well as normal colonic cells (positive control) were positive. Normal liver tissue was used as a positive control for each TMA. We used an evaluation score of the loss of SMARCB1 in 3 categories according to the percent of positive tumor cells [19], and 2 subcategories: focal or diffuse loss. We only considered the percentage of labeled cells with SMARCB1 antibody, and not the intensity of the marking (Fig. 1 and Supplementary file 1). The score was noted "not interpretable" when neoplastic cells and also internal controls were negative for SMARCB1.

For the first series of patients, IHC of all cases was performed on tissue micro array (TMA) sections, and those who had focal or diffuse total loss of SMARCB1 underwent staining on whole sections. Immunohistochemistry on whole sections was interpreted as diffusely negative when there was negative staining in all neoplastic cells and a positive control in non-neoplastic cells (Fig. 2).

We then analyzed the expression of SMARCB1 in whole sections in the small independent cohort of

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Fig. 1. SMARCB1 immunohistochemistry on TMA of colon carcinoma. A: More than 90% of tumor cells were positive (score "3d"). *Of note, neoplastic and non-neoplastic cells had nuclear expression of SMARCB1*, because the expression of SMARCB1 is ubiquitous in non-tumor cells. B: 51 to 90% of tumor cells were positive with a diffuse distribution of loss of SMARCB1 (score "2d"). C: 5 to 50% of tumor cells were positive with a diffuse distribution of loss of SMARCB1 (score "2d"). C: 5 to 50% of tumor cells were positive with a diffuse distribution of loss of SMARCB1 (score "2d"). D: 51 to 90% of tumor cells were positive with a focal or multifocal distribution of loss of SMARCB1 (score "2f"). *Note that a loss is said to be "focal" if there is a loss of SMARCB1 expression on at least 25% of the TMA spot and if possible on more than one TMA spot out of 4*. E: 5 to 50% of tumor cells were positive with a focal distribution of loss of SMARCB1 (score "1f"). F: Less than 5% tumor cells were positive with a focal distribution of loss of SMARCB1 (score "1f"). F: Less than 5% tumor cells were positive with a focal distribution of loss of SMARCB1 (score "0f"). G: Less than 5% of neoplastic cells are negative as opposed to lymphoid stroma. The non-neoplastic cells retain nuclear expression of SMARCB1 and act as an internal positive control (score "0d").



Fig. 2. Kaplan-Meir survival curves comparing SMARCB1 negative (group 3) and SMARCB1 positive CC (groups 1 and 2). A: Recurrence-Free survival according to SMARCB1 status. B: DFS according to SMARCB1 status. C: Overall survival according to SMARCB1 status.

23 patients treated in Boulogne or Nantes for poorly 95

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2.3. Statistic analyses 97

Prognostic and molecular status (mismatch repair 98 (MMR) and BRAF V600E mutation) and detailed 99 methods used to determine this status have been pre-100 viously reported in the literature [4,14,15]. The asso-101 ciation between CC clinicopathological and molecu-102 lar variables and SMARCB1 expression were individ-103 ually examined and binary logistic regression model-104 ing was used. Kaplan Meier analysis and Cox regres-105 sion modeling were employed to examine the impact 106 of SMARCB1 expression with overall survival. A p <107 0.05 was taken as significant. For statistical analyzes 108 we used 3 groups as follows: 109 210 110

	Group 1:	3d (conserved expression of SMARCB1
		on more than 90% of tumor cells)
2	Group 2:	1 and 2 (labeling of SMARCB1 on 5 to
3		90% of tumor cells)
Ļ	Group 3:	0 (total loss of SMARCB1)
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2.4. SMARCB1 molecular analysis

Tumor DNA was extracted from FFPE samples af-116 ter histology control and selection of areas contain-117 ing more than 80% of tumor cells. The whole coding 118 sequence as well as the previously described hotspot 119 in the first intron of SMARCB1 were analyzed us-120 ing next generation sequencing (NGS). Libraries ob-121 tained with Agilent SureSelect XT-HS preparation kit 122 were sequenced on a NovaSeq 6000 system. The bioin-123 formatics analysis included a variant calling using 124 Varscan2 (v2.4.3) and TransIndel for intermediate insertion/deletion and a copy number profile using Facets (v0.5.1) with a sex-match control as reference [10,16]. 127

3. Results

SMARCB1 immunostaining on TMA was inter-129 pretable in 1695 out of 2043 (82.9%) patients with 130 stage III CC of the PETACC8 trial. In the 1695 (18.6%) 131 analyzed cases, 315 were poorly or undifferentiated 132

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differentiated CC. 96

		Group 1 ($N = 1235$)	Group 2 (N = 448)	Group 3 $(N = 12)$	Total $(N = 1695)$	<i>p</i> -value
Gender	n	1235	448	12	1695	X ² : 0.0759
	Male	688 (55.7%)	274 (61.2%)	5 (41.7%)	967 (57.1%)	
	Female	547 (44.3%)	174 (38.8%)	7 (58.3%)	728 (42.9%)	
Age (Class)	n	1235	448	12	1695	X ² : 0.5896
	≤ 70 ans	1108 (89.7%)	407 (90.8%)	10 (83.3%)	1525 (90.0%)	
	> 70 ans	127 (10.3%)	41 (9.2%)	2 (16.7%)	170 (10.0%)	
Age	n	1235	448	12	1695	KW: 0.156
	Moy (SD)	59.06 (9.54)	59.91 (9.23)	54.92 (14.08)	59.26 (9.50)	
	Médiane	60.00	62.00	59.50	60.00	
	Q1; Q3	53.00; 67.00	54.00; 67.00	46.00; 64.50	54.00; 67.00	
	Min; Max	23.00; 75.00	19.00; 75.00	28.00; 72.00	19.00; 75.00	
VHO performance	n	1190	436	11	1637	X ² : 0.816
tatus	0	966 (81.2%)	354 (81.2%)	10 (90.9%)	1330 (81.2%)	
	1	218 (18.3%)	82 (18.8%)	1 (9.1%)	301 (18.4%)	
	2	5 (0.4%)	0 (0.0)	0 (0.0)	5 (0.3%)	
	3	1 (0.1%)	0 (0.0)	0 (0.0)	1 (0.1%)	
ocalisation	n	1231	444	12	1687	X ² : 0.0624
	Left localization	711 (57.8%)	291 (65.5%)	7 (58.3%)	1009 (59.8%)	
	Right localization	506 (41.1%)	147 (33.1%)	5 (41.7%)	658 (39.0%)	
	Both sides	14 (1.1%)	6 (1.4%)	0 (0.0)	20 (1.2%)	
Bowel obstruction	n	1235	448	12	1695	X ² : 0.550
nd perforation	Bowel obstruction and/or perforation	234 (18.9%)	90 (20.1%)	1 (8.3%)	325 (19.2%)	
	No bowel obstruction and no perforation	1001 (81.1%)	358 (79.9%)	11 (91.7%)	1370 (80.8%)	
Iistopathology	n	1234	448	12	1694	X ² : 0.774′
rading	Well differentiated	246 (19.9%)	102 (22.8%)	0 (0.0)	348 (20.5%)	
	Moderately differentiated	743 (60.2%)	261 (58.3%)	8 (66.7%)	1012 (59.7%)	
	Poorly differentiated	227 (18.4%)	77 (17.2%)	4 (33.3%)	308 (18.2%)	
	Undifferentiated	5 (0.4%)	2 (0.4%)	0 (0.0)	7 (0.4%)	
N classification	n	1235	448	12	1695	X ² : 0.0143
	pN1	783 (63.4%)	260 (58.0%)	11 (91.7%)	1054 (62.2%)	
	pN2	452 (36.6%)	188 (42.0%)	1 (8.3%)	641 (37.8%)	
T classification	\overline{n}	1235	448	12	1695	X ² : 0.6254
	pT1	30 (2.4%)	13 (2.9%)	0 (0.0)	43 (2.5%)	
	pT2	81 (6.6%)	30 (6.7%)	2 (16.7%)	113 (6.7%)	
	pT3	870 (70.4%)	301 (67.2%)	9 (75.0%)	1180 (69.6%)	
	pT4	253 (20.5%)	103 (23.0%)	1 (8.3%)	357 (21.1%)	
	pTis	0 (0.0)	1 (0.2%)	0 (0.0)	1 (0.1%)	
	рТх	1 (0.1%)	0 (0.0)	0 (0.0)	1 (0.1%)	
/ELI	n	1235	448	12	1695	X ² : 0.2864
	Vascular invasion or lymphatic infiltration	685 (55.5%)	265 (59.2%)	6 (50.0%)	956 (56.4%)	
	No vascular invasion and no lymphatic	350 (28.3%)	128 (28.6%)	3 (25.0%)	481 (28.4%)	
	infiltration					
	Missing	200 (16.2%)	55 (12.3%)	3 (25.0%)	258 (15.2%)	
Combined	n	1151	407	9	1567	$X^2: 0.1416$
RAS/BRAF	Double WT	448 (38.9%)	180 (44.2%)	2 (22.2%)	630 (40.2%)	
	RAS mutant	569 (49.4%)	183 (45.0%)	7 (77.8%)	759 (48.4%)	
	BRAF mutant	134 (11.6%)	44 (10.8%)	0 (0.0)	178 (11.4%)	
AS	n	1148	405	9	1562	$X^2: 0.065$
	Wild-Type	578 (50.3%)	222 (54.8%)	2 (22.2%)	802 (51.3%)	
	Mutated	570 (49.7%)	183 (45.2%)	7 (77.8%)	760 (48.7%)	
BRAF	n	1182	426	10	1618	$X^2: 0.0000$
	Wild-Type	1045 (88.4%)	378 (88.7%)	8 (80.0%)	1431 (88.4%)	
	Mutated	117 (9.9%)	41 (9.6%)	0 (0.0)	158 (9.8%)	
	Test failure	20 (1.7%)	7 (1.6%)	2 (20.0%)	29 (1.8%)	
/IMR status	n	1235	448	12	1695	$X^2: 0.0699$
	pMMR	1103 (89.3%)	416 (92.9%)	10 (83.3%)	1529 (90.2%)	
	dMMR	132 (10.7%)	32 (7.1%)	2 (16.7%)	166 (9.8%)	

CC. There was no significant difference in outcome according of histological grade (Table 1).

Tumors from 12 of these patients (group 3 = 0.7%) were had either focal (n = 9) or diffuse (n = 3) total loss of SMARCB1 on TMA and underwent IHC on whole sections. These tumors were all moderately (8/12) or poorly (3/12) differentiated. Whole section controls disclosed a complete loss in only one case, corresponding to a medullary carcinoma.

The clinicopathological and molecular features of
the CC with and without SMARCB1 loss are presented in Table 1. Diffuse or focal SMARCB1 loss was
not associated with clinical characteristics, histological grade, tumor size, mismatch repair deficiency nor *BRAF* mutation.

Loss of SMARCB1 was not associated with poorer
 survival (Supplementary file 2, Fig. 2).

Surprisingly, group 1 (with a conservation of
 SMARCB1 expression) had a worse node status. But
 this is irrelevant clinically and is probably due to a bias
 related to the low number of cases in group 3.

Among the 23 poorly or undifferentiated CC of the second cohort, we detected 2 (8.7%) cases with total focal loss of SMARCB1; one was rhabdoid and the other poorly differentiated (medullary and mucinous). These 2 cases were deficient for MMR and mutated for *BRAF* (Supplementary files 3 and 4).

Molecular mechanisms responsible for the loss of 160 SMARCB1 expression were investigated in the two 161 cases with total and focal loss. NGS allowed a mean 162 coverage of 699X and 488X over the whole design in 163 those two cases and a minimal coverage of 100X for 164 SMARCB1, but did not reveal any pathogenic variant 165 in the coding sequence, nor at the hotspot within intron 166 1 of SMARCB1. Copy number profile also assessed by 167 NGS revealed neither large deletion nor duplication in 168 SMARCB1. Interestingly, we detected a large region of 169 copy-neutral loss of heterozygoty (LOH) encompass-170 ing the whole SMARCB1 locus in one of the cases. 171

172 **4. Discussion**

We detected a loss of SMARCB1 in 12 out 1695 173 (0.7%) cases with stage III CC included in the prospec-174 tive international clinical trial PETACC8 and did not 175 found any correlation with clinical, histologic or 176 molecular characteristics, nor with survival. The fre-177 quency of loss of SMARCB1 was higher in our second 178 cohort of 23 patients with poorly differentiated CC. 179 The incidence of loss of SMARCB1 in CC was 180

initially reported to be 11% (15/134) [11]. However,

in a very large series of 3041 unselected CC, Wang 182 et al. detected only 14 (0.46%) cases with loss of 183 SMARCB1 [19], which was significantly different 184 from the first report (p < 0.001, Xhi2 test). We use the 185 same evaluation score as Wang et al. and in our main 186 series of patients (cohort 1), which is limited to patients 187 with stage III CC, the frequency of loss of SMARCB1 188 was not different from Wang et al. (p = 0.2, Xhi2 test),189 suggesting that the stage of CC has no or only limit cor-190 relation with this phenotype. Altogether in these three 191 largest published series of CC, the frequency of loss of 192 SMARCB1 was 0.84% in 4880 patients, which likely 193 reflects a real incidence below 1% in CC of any stage. 194

The frequency of loss of SMARCB1 seems to be 195 higher in poorly differentiated CC. In fact, in the Italian 196 series, 8 cases with loss out of 25 poorly differentiated 197 CRC (32%) were observed. Among the 511 high-grade 198 cases of Wang, 12 were SMARCB1 negative (2.35%). 199 Briefly in Wang series, 12 of 14 (85.7%) of the CRCs 200 with SMARCB1 loss were high grade, compared to 201 19.6% of cases with preserved SMARCB1 staining 202 (p < 0.001). In 3 of the 7 CRCs with focal SMARCB1 203 loss, SMARCB1 stained in well-differentiated areas, 204 and lost in areas of poor differentiation. In our large 205 series of stage III CC, the only case with complete loss 206 was a poorly differentiated CC: medullar type. Further-207 more in our small independent series of poorly differ-208 entiated CC: 2/23 (8.7%) were SMARCB1 negative. 209

Although originally described in malignant rhab-210 doid pediatric tumors, SMARCB1 loss has now been 211 reported in tumors from several localization, includ-212 ing the vulva [8], pancreas [2] and sinonasal tract [3]. 213 In these sites, as well as in the gastrointestinal tract, 214 SMARCB1 loss was reported to be associated with 215 poor prognosis [1,11,17,19]. For instance, among the 216 134 cases of Pancione et al. the loss was associ-217 ated with poorly differentiated tumors, most often 218 metastatic, and with a lower survival, regardless of the 219 MMR status. Wang et al. also observed that loss of 220 SMARCB1 was associated with lower survival. Con-221 trasting with these previous publications, we did not 222 find any prognostic value of the loss of SMARCB1. 223

The previous studies retrospectively included pa-224 tients with different UICC stages, and with heteroge-225 neous treatment and follow up. By contrast, the 1695 226 that we analyzed were all at stage III, prospectively 227 included in an international clinical trial. They all re-228 ceived FOLFOX adjuvant treatment and underwent the 229 same follow up. For these reasons our results have a 230 higher level of evidence, than the previously published 231 series. However, the power of our series is limited by 232

the low incidence of the SMARCB1 negative phenotype.

Only few cases of CC with rhabdoid histology and 235 loss of SMARCB1 have been reported as yet. But they 236 had common features: mean age 70 years old, localiza-237 tion of the proximal colon, metastases, poor prognosis, 238 BRAF mutated and dMMR status [1,12]. Wang's group 239 showed that the loss of SMARCB1 in CC was asso-240 ciated with MSI-H status, and BRAF V600E mutation 241 (p < 0.001). Molecular characteristics of the tumors 242 of patients included in the PETACC8 series have al-243 ready been published [4,14,15]. In these patients, loss 244 of SMARCB1 was not associated with mismatch re-245 pair deficiency nor with BRAF V600E mutation. How-246 247 ever, in our small series of poorly differenciated CC, one of the two cases with loss was rhabdoid, the other 248 mixed (medullary and mucinous) and these 2 cases 249 were BRAF mutated and deficient for MMR. We can 250 therefore speculate that SMARCB1 loss could be sec-251 ondary to genetic instability. However no pathogenic 252 somatic variant were detected within the coding se-253 quences. The previously described hotspot mutation within intron 1 may also be responsible for the loss of 255 SMARCB1 [16], but were also absent in the two cases. 256 By contrast we detected a copy neutral LOH in one of 257 the two cases. Further studies are required to determine 258 the mechanism of SMARCB1 loss in poorly differen-259 tiated gastrointestinal carcinoma. 260

Genes of the SWI/SNF chromatin-remodeling complex are frequently altered in human cancers [20] and may be targeted by specific therapies in the future.

To date, there is no available targeted molecular 264 therapy against pediatric rhabdoid neoplasms, but cur-265 rently a recently developed EZH2 inhibitor, is un-266 dergoing clinical trial in children with rhabdoid tu-267 mor and loss of SMARCB1 (NCT02601937). EZH2 268 is a catalytic subunit of the histone methytransferase 269 PCR2 which is blocked by the intact SWI/SNF com-270 plex [17]. When SMARCB1 is mutated, the accumu-271 lation of EZH2 promotes an undifferentiated state with 272 maintenance of a "stem cell" program. Hedgehog-Gli 273 pathway, Cyclin D1, Epidermal growth factor and Fi-274 broblastic growth factor receptors [21] are other poten-275 tial targets, which have been found to be up regulated 276 in association with a disrupted SWI/SNF complex. 277

In view of the results obtained in the literature and in the independent cohort of undifferentiated CC studied in parallel, it seems advisable to evaluate the prognostic impact of the loss of expression of SMARCB1 by restricting the studied population to the poorly dif-

²⁸³ ferentiated CC. The IHC could allow a pre-screening

of mutated patients, who may one day benefit from targeted therapies.

In conclusion, loss of SMARCB1 expression is rare (< 1%) in stage III CC, but more frequent (> 5%) in poorly differentiated CC. Our study did not confirm the association of loss of SMARCB1 with MMR nor *BRAF* status, neither with poorer prognostic.

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Supplementary data

The supplementary files are available to download from http://dx.doi.org/10.3233/CBM-190287.

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