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ORIGINAL ARTICLE

Expression of the Serrated Markers Annexin A10 or Gremlin1 in Colonic Adenocarcinomas: Morphology and Prognostic Values

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Abstract

Describe clinical, histological and molecular charatcteristics and prognosis values of the serrated candidate markers AnnexinA10 and Gremlin1 in colon adenocarcinomas. Immunohistochemical expression of AnnexinA10 and Gremlin1 was evaluated on 346 colonic adenocarcinomas. Clinicopathological, molecular features and prognostic characteristics were then evaluated. A total of 40 colonic adenocarcinomas expressed AnnexinA10 (11.6%) and, 115 expressed Gremlin1 (40.4%). AnnexinA10 expression was significantly associated, on univariate analyses, with female gender ($p = 0.03$), right tumor location ($p < 0.001$), differentiation grade 3 ($p < 0.001$), serrated adenocarcinoma subtype ($p < 0.001$), serrated ($p < 0.001$), medullary ($p = 0.005$), and mucinous component ($p = 0.004$), cytoplasmic eosinophilia ($p < 0.001$), discernible nuclei ($p = 0.001$), preserved polarity ($p < 0.001$), lymphatic invasion ($p = 0.01$), *BRAFV600E* mutation ($p < 0.001$), MSI-H status ($p < 0.001$) and CIMP-H status ($p = 0.019$). Multivariate analyses revealed that mucinous component ($p = 0.002$), lymphatic invasion ($p = 0.02$) and *BRAFV600E* mutation ($p < 0.001$) were independently associated with AnnexinA10 expression. In addition, AnnexinA10 was an indicator of poorer overall survival (OS) in UICC stage IV adenocarcinomas ($p = 0.01$) only. Gremlin1 expression was neither associated with serrated adenocarcinoma subtype ($p = 0.51$) nor with AnnexinA10 expression ($p = 0,31$), but was significantly associated, in univariate analysis with male gender ($p = 0.002$), younger age ($p = 0.002$), left tumor location ($p = 0.04$), and MSS status ($p = 0.03$). Gremlin1 expression was associated with better OS only in UICC stage III colon adenocarcinomas ($p = 0.006$). Colon adenocarcinomas expressing AnnexinA10 have distinct clinico-pathological and molecular features. AnnexinA10 expression is an indicator of poorer OS in UICC stage IV patients. Gremlin1 expression is not associated with serrated adenocarcinomas subtype. Its expression was associated with better OS in UICC Stage III patients.

Keywords Colorectal neoplasms · AnnexinA10 · Gremlin1 · Prognosis · Serrated adenocarcinoma

Introduction

Colorectal cancer (CRC) is the third most common type of cancer and the second leading cause of cancer death worldwide [1]. Most CRC develop from conventional adenomas according to the adenoma-carcinoma sequence involving *APC*, *KRAS* and *p53* mutations. In contrast, a minority of CRC (7.5%) recently called serrated adenocarcinomas (SAC), develop from serrated polyps on the basis of an alternative pathway of CRC. This pathway, known as the serrated pathway is characterized by molecular alterations such as activation of the MAPK pathway through mutations of *BRAF* or *KRAS*, high CpG island methylator phenotype (CIMP-H) and low or high-level DNA microsatellite instability (MSI-L/H) [2, 3].

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Tumors of serrated pathway occur more frequently in women, are discovered at an advanced age and are mainly located in the right colon [2]. Diagnostic criteria for SAC, according to the World Health Organization (WHO) are: "morphological similarities with serrated polyps, with glandular serration that can be accompanied by mucinous areas. The tumors cells have a low N:C ratio" [4]. A previous study specified that the diagnosis of SAC can be based on the following criteria: abundant eosinophilic cytoplasm, easily discernible nuclei with chromatin condensation around the nuclear envelope, preserved polarity, and architecture described as being serrated in differentiated carcinomas, trabecular in poorly differentiated carcinomas, and mucinous in mucin-producing carcinomas [5]. Molecularly, SAC are more often associated with CIMP-H, MSI-H status, and *BRAFV600E* mutation. *KRAS*-mutated adenocarcinomas have also been described, showing concurrent loss of *MGMT* and functionally preserved *APC* [3].

Thus, in current practice, the diagnosis of SAC can be challenging because the histological criteria remain imprecise, especially in poorly differentiated cases.

Recently, Sajanti et al. [6] identified Annexin A10 (ANXA10), as a potential marker of the serrated pathway and especially sessile serrated lesion and SAC. ANXA10 thus, could clinically help in rare difficult cases to distinguish sessile serrated lesion from conventional adenoma.

ANXA10 is a member of the annexin family, a large multigene family of calcium and phospholipid-binding proteins with diverse range of cellular functions such as: vesicle trafficking, cell division, apoptosis, calcium signaling and growth regulation [7]. The role of ANXA10 in tumorigenesis remains incompletely defined. Its expression would have a different prognostic value depending on the primitive site. Some authors have reported that low expression of ANXA10 was associated with poor survival in gastric and bladder cancer [8, 9], as well as early recurrence and poor prognosis in hepatocellular carcinoma [10]. Whereas, high ANXA10 expression in oral squamous cell carcinomas was associated with advanced-tumors stage and was correlated with tumor size [11].

The prognosis impact of ANXA10 in CRC was assessed in one study only [12]. This study showed that ANXA10 was associated with poor overall survival and progression free survival, especially in stage IV CRC.

According to the literature, ANXA10 is overexpressed in 5.8 to 8.8% of all CRC [12], and in 17 to 33% of sporadic MSI-H CRCs [13, 14]. In addition, CRC overexpressing ANXA10 have the same clinical and molecular characteristics as SAC: women predominance, proximal tumor location, and MSI-H, CIMP-H and *BRAF*-mutated molecular statuses [6, 12–14]. In these studies, cancers expressing ANXA10 had a serrated morphology, without any detail of cytological aspects.

Another potential marker of serrated pathway, Gremlin1, a product of *GREM1* gene, has also been recently identified [15]. Abundant Gremlin1 expression seems to be associated with serrated histology, low histological grade, low TNM stage and would also be a stage independent indicator of better survival. No morphological criteria other than serrated architecture were analyzed. Gremlin1 plays a role in regulating organogenesis, tissue differentiation and angiogenesis. A recent study shows a potential role of Gremlin1 as a better prognosis indicator in gastric cancer [16]. To date Gremlin1 immunolabelling doesn't have any established routine use.

The aims of our study are to thoroughly describe the histological features of CRC expressing the serrated markers ANXA10 or Gremlin1 and to evaluate the prognosis impact of their expression.

Materials and Methods

Patients

This study was based on adult patients who underwent surgery for sporadic colon cancer in the Digestive Surgery Department of the Academic Hospital of Reims, between September 2006 and December 2012. Patients with rectal cancer or who underwent neoadjuvant therapies were excluded. All patients had given their consent for biospecimen use and the study was performed in accordance with the ethical standards laid down in the Declaration of Helsinki. The written consent of patients to the biospecimen use was obtained in all cases. Approval for the study was previously obtained from the local Institutional Review Board and the Tissue Bank Management Board.

Clinical information including sex, age at the time of surgery, tumor location, surgical circumstances (tumor perforation, occlusion), tumor recurrence, death, follow-up information were retrieved from medical records. Patients were classified as having a right colonic cancer if the primary tumor was located in the caecum, ascending colon, hepatic flexure or transverse colon, and left colonic cancer if the tumor site was within the splenic flexure, descending colon, sigmoid colon or rectosigmoid junction. Molecular data including microsatellite instability (MSI) status, CIMP status, *RAS* and *BRAF* status were also collected.

Pathology

All cases of colon adenocarcinomas were classified and subtyped according to The World Health Organization criteria [4] and staged according to the International Union Against Cancer 2009 guidelines [17]. All slides were retrieved from the archives of the Department of Pathology of the Academic Hospital of Reims and were reviewed and classified by three pathologists (BM, CBR and MDD). Adenocarcinomas were

classified as non serrated, serrated or potential serrated according to The World Health Organization and Tupurrainen criteria [4, 5]. The category of "potential serrated carcinomas" corresponded to adenocarcinomas with suggestive characteristics of SACs (not all criteria were present). These criteria were evaluated blinded to ANXA10 and Gremlin1 immunohistochemical results. Tumor budding was assessed on Hematoxylin- Eosin-Saffron slides as previously described [18].

The following morphological criteria were evaluated for all ANXA10 positive cases and the same number of randomly selected ANXA10 negative cases: percentage of serrated, mucinous, cribriform, tubulo-papillary, micropapillary or medullary architecture, tumor necrosis percentage, tumor stroma percentage, degree of stromal inflammation (0: absent, 1: mild, 2: moderate, 3: abundant), presence or absence of tumor infiltrating lymphocyte (TIL), type of tumor invasion front (expansive, invasive type or both), presence of a serrated polyp at the edge of the tumor, presence and percentage of eosinophilic cytoplasms, vesicular nuclei, discernible nuclei, prominent nucleoli, nuclear stratification and presence of brush border at the apex of the cells, degree of cytonuclear atypia, perineural, lymphatic or vascular invasion and tumor budding.

Tissue Microarray (TMA) Construction and Immunohistochemistry

All tissue samples were analyzed via tissue microarrays (TMA). For each tumor, 3 cores were punched in the central part and 3 cores at the invasive front of the tumor from the same original formalin-fixed paraffin-embedded tumor block. In some TMA, a core of normal colonic mucosa was inserted between each tumor cases ($n = 161$). The cores were precisely arrayed into a recipient paraffin block using the MiniCore Tissue Arrayer (Excilone, Elancourt, France). Sections of 4-μm thickness were cut and mounted on SuperFrost Ultra Plus Gold adhesive slides (ref. 11,976,299, Thermofisher Scientific, Waltham, MA, USA). Then, immunohistochemistry was performed using anti-ANXA10 (1/400, rabbit polyclonal, ref. NBP1–90156, Novus Biologicals, Littleton, CO, USA) and anti-Gremlin1 antibodies (1/50, rabbit polyclonal, ref. ab22138, Abcam, Cambridge, United Kigdom) with the BenchMark XT automated slide stainer (Ventana Medical Systems, Tucson, AZ, USA). CDX2, MLH1, PMS2, MSH2 and MSH6 immunohistochemistry were performed as previously described [19, 20].

ANXA10, Gremlin1, CDX2, MLH1, MSH2, MSH6 and PMS2 immunolabelling were evaluated in a binary manner as either positive or negative independently by 3 pathologists (AMB, BM, CB-R). For ANXA10 and Gremlin1 tumor cell positivity percentage evaluation, the mean score of all the TMA core percentage of each case was used in order to closely approach the whole slide type evaluation. All tumors in which the tumor nucleus either completely lacked immunostaining or showed staining in a minority (<1%) of tumor cells for ANXA10 were scored as negative. Immunohistochemical findings were considered as positive when at least 1% of the tumor cells showed an unequivocal nuclear staining. Gremlin1 cytoplasmic expression was scored identically when unequivocal cytoplasmic expression was observed in at least 1% of malignant cells. In case of discrepancies, a consensus diagnosis was reached. For cases with missing cores or absence of tumor cells a whole-tissue section was performed.

Molecular Analyses

Tumor DNA was extracted from formalin-fixed, paraffin-embedded (FFPE) tissues after macrodissection of a preselected area with the highest adenocarcinomatous cells density. Each sample analyzed contained at least 30% of tumor cells. DNA was extracted on Maxwell® 16 Instruments automaton after tumor macrodissection with Maxwell® 16 FFPE Tissue LEV DNA Purification Kit (all products from Promega Corporation, Madison, USA). *BRAF* mutation detection was assessed by allelic discrimination using TaqMan probes as previously described [19]. *KRAS* gene mutations on codons 12 and 13 were searched by pyrosequencing analysis using the Pyromark Q96 kit (Quiagen GmbH, Hilden, Germany) as previously described [19]. The MSI status was determined by Multiplex PCR analysis of 5 mononucleotitic markers (BAT25, BAT26, MONO27, NR21, NR24) and 2 pentanucleotidic markers (PentaC and Penta D) using the MSI Analysis System kit, Version 1.2 (ref. MD1641, Promega Corporation, Madison, USA) as previously described [19].

Statistical and Survival Analyses

Data were described using mean and standard deviation for quantitative variables and number and percentage for qualitative variables. Factors associated with ANXA10 or Gremlin1 expression, and the morphological classification (non serrated, serrated or potential serrated) were studied using univariate analyses (Chi2 tests, Fisher's exact tests, Student's *t* tests, linear regressions or Wilcoxon tests, as appropriate) and multivariate analysis (logistic regressions with stepwise selection, with entry and removal limits set at 0.10 and factors significant at $p = 0.10$ in univariate analysis included). Overall and event-free survivals were studied. In event-free survival, event considered were disease progression or recurrence. The survival curves were established by the Kaplan-Meier method. For each analysis, prognostic factors were identified by univariate analysis using Log rank tests and by multivariate analysis using a Cox proportional hazard model. Factors significant at the 0.10 level in univariate analysis were included in a

stepwise regression multivariate analysis with entry and removal limits set at 0.10. Statistical analyses were performed with SAS version 9.4 (SAS institute Inc., Cary, North California). For all tests, $p < 0.05$ were considered to be statistically significant.

Intra- and inter-observer variation was quantified using the unadjusted kappa coefficient of agreement (κ).

Results

Patients and Clinicopathological Features

A total of 346 patients were included in our study. The male to female ratio was 1.35:1 (199 males (57.5%) and 147 females (42.5%)) and mean age was 70.6 years ±11.2 years. Tumors were right-sided in 154 cases (44.5%) and left-sided in 192 cases (55.5%). Nine cases (2.6%) were classified as serrated adenocarcinoma and 10 cases (2.9%) were classified as potentially serrated adenocarcinoma. Follow-up data were available for all except 12 patients. The median follow-up time was 43 months [minimum: 1 day – maximum: 111 months]. Clinicopathological features of the cohort are detailed in Table 1.

Table 1 Clinicopathological features of the cohort ($n = 346$)

Clinical/pathological features	n (%)
Gender	
Male	199 (57.5)
Female	147 (42.5)
Age (Mean ± standard deviation) years	70.6 ± 11.2
UICC stage	
Stage I	40 (11.7)
Stage II	133 (38.8)
Stage III	85 (24.8)
Stage IV	85 (24.8)
Tumor location	
Left colon	192 (55.5)
Right colon	154 (44.5)
Differentiation grade	
Grade 1–2	291 (84.1)
Grade 3	55 (15.9)
Annexin A10	
Positive	40 (12.7)
Negative	276 (87.3)
Gremlin1	
Positive	115 (40.3)
Negative	170 (59.7)
Serrated adenocarcinoma	
Yes	9 (2.6)
No	327 (94.5)
Potential	10 (2.9)
KRAS status	
Wild type	108 (69.2)
Mutant	48 (30.8)
BRAF status	
Wild type	265 (85.8)
Mutant	44 (14.2)
Microsatellite status	
MSS	274 (87)
MSI	41 (13)
CIMP status	
No CIMP	23 (37.1)
CIMP-Low	32 (51.6)
CIMP-High	7 (11.3)

Clinico-Pathological Characteristics of ANXA10+ and Gremlin+ Ecolonic Adenocarcinomas

ANXA10 and Gremlin Expression in the CRC Cohort

ANXA10 immunohistochemical expression analysis had an almost perfect interobserver agreement (κ between 0.91 and 0.96). Discordant cases were collegially reviewed and a total of 40 cases (12.7%) of adenocarcinomas expressing ANXA10 were retained The percentage of ANXA10 positive tumor cells nucleus was 12.2 ± 12.8 [1–65%]). Thirteen of these 40 cases (32,5%) had global ANXA10 nuclear positivity percentage between 1 and 5% and 27/40 (67.5%) expressed ANXA10 in more than 5% of the tumor cells. All cases with a global positivity score between 1 and 5% had at least one TMA core with a positivity score ≥ 5%. In normal colonic mucosa ($n = 161$), a weakly cytoplasmic ANXA10 expression was observed in epithelial cells, but no nuclear expression. Some inflammatory cells of the lamina propria showed ANXA10 cytoplasmic and nuclear expression. Examples of ANXA10 immunolabelling are given in Fig. 1a, b.

Gremlin1 expression was successfully evaluated on 285/346 cases. The inter-observer agreement was perfect (no discrepancy). A total of 115 cases expressed Gremlin1 (40.4%). Gremlin1 immunolabelling was cytoplasmic and granular. The percentage of tumor cells expressing Gremlin1 varied between 1 and 82% (mean of 23.5% ± 23.4). No expression was found in normal colonic mucosa ($n = 161$). Examples of Gremlin immunolabelling are given in Fig. 1c, d.

Only 11 cases co-expressed Gremlin1 and ANXA10. Expression of these two markers were not statistically correlated ($\kappa = -0.045$; $p = 0.31$).

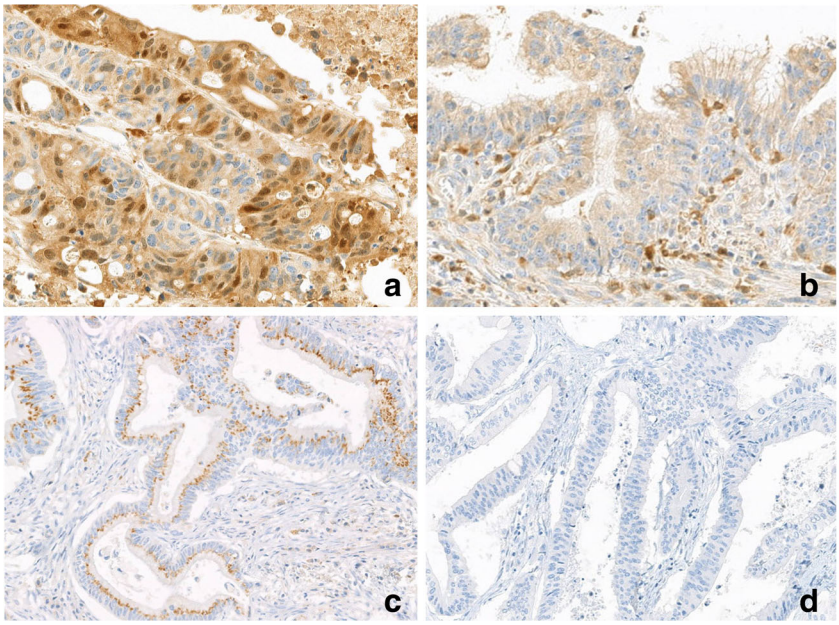

Fig. 1 Examples of AnnexinA10 and Gremlin1 immunohistochemical expression in colon adenocarcinoma and mucosa. **a** A case of AnnexinA10 expressing colon adenocarcinoma (×40 magnification). Strong nucleus expression of AnnexinA10 in malignant cells was associated with moderate cytoplasmic expression. **b** A case of AnnexinA10 negative colon adenocarcinoma (×40 magnification). Some stromal cells expressed AnnexinA10 with a cytoplasmic positivity only. **c** An example of Gremlin1 positive colon adenocarcinoma (×20 magnification). Gremlin1 showed a granular cytoplasmic immunostaining in malignant cells only. **d** A case of Gremlin1 negative colon adenocarcinoma (×20 magnification)

Histological Characteristics of ANXA10+ CRC

The 40 ANXA10 expressing cases were compared to 40 randomly selected ANXA10 negative cases. Description of the 80 selected cases is reported in *Supplementary table*.

Univariate and multivariate analysis results comparing ANXA10+ and ANXA10- cases pathological characteristics are detailed in Table 2. ANXA10 expression was significantly associated on univariate analyses with serrated architecture ($p < 0.001$) (Fig. 2a, b), medullary pattern ($p = 0.005$) (Fig. 2c), and mucinous pattern ($p = 0.004$) (Fig. 2d). In univariate analysis, ANXA10+ CRC had significantly more cytoplasmic eosinophilia ($p < 0.001$), more discernible nuclei ($p = 0.001$), less preserved polarity ($p < 0.001$), less well-ordered brush border ($p = 0.005$), less abundant tumor stroma ($p = 0.02$) and more frequent lymphatic invasion ($p = 0.01$). Nine ANXA10+ cases (22.5%) were classified as serrated carcinomas and 10 (25.0%) as potential serrated adenocarcinomas (Fig. 2e). No ANXA10 negative case was classified in both categories ($p < 0.001$). A total of 15 ANXA10+ cases (37.5%) totally lacked both serrated architecture and cytoplasmic eosinophilia that are the characteristical criteria of serrated adenocarcinomas. Thus, one third of ANXA10+ adenocarcinoma totally lacked the typical serrated morphology. Among these cases, 5 were of medullary and 3 mucinous subtypes of adenocarcinomas, and the 7 other had classical colon adenocarcinoma morphology. We found a sensitivity of 100% and a specificity of 56% for ANXA10 expression as marker for SAC.

In multivariate analysis (Table 2), colon adenocarcinomas expressing ANXA10 were significantly associated with mucinous architecture (OR = 7.2 [2.1–27.8]; $p = 0.002$) and lymphatic invasion (OR = 4.3 [1.3–14.9]; $p = 0.02$) and showed a tendency to be associated with cytoplasmic eosinophilia (OR = 1.03 [0.99–1.06]; $p = 0.08$) and discernible nuclei (OR = 1.05 [0.99–1.09]; $p = 0.055$). Because of multicollinearity, serrated architecture and serrated classification have not been included in multivariate analysis.

Clinicopathological and Molecular Characteristics of ANXA10+ Cases

The relationship between ANXA10 expressing adenocarcinomas and different clinicopathological characteristics was analyzed. Results are reported in Table 3. ANXA10 expression was significantly associated on univariate analysis, with female gender ($p = 0.03$), right tumor location ($p < 0.001$), poor differentiation grade ($p < 0.001$), absence of CDX2 expression ($p = 0.007$), *BRAF* mutation ($p < 0.001$), MSI-H status ($p < 0.001$) and CIMP-H status ($p = 0.019$). In multivariate analyses, colon adenocarcinomas expressing ANXA10 were independently associated with *BRAF* mutation only ($p < 0.001$).

Table 2 Relationships between ANXA10 expression and histological features in colonic cancers

Histological variables	Annexin A10		Univariate analysis	Multivariate analysis	
	Positive ($n = 40$) n (%) or mean ± SD	Negative ($n = 40$) n (%) or mean ± SD	*p*	OR [IC 95%]	*p*
Serrated architecture	17 (42.5)	0 (0)	<0.0001 ‡		NA
Tubulo-papillar pattern	37 (92.5)	39 (97.5)	0.62 †		
% tubule-papillar pattern	47.8 ± 31.5	73.7 ± 29.1	0.001		
Cribriform pattern	27 (67.5)	27 (67.5)	1.000 ‡		
% cribiform pattern	20.7 ± 23.5	18.8 ± 19.9	0.88		
Mucinous pattern	25 (62.5)	12 (30.0)	0.004 ‡	7.3 [2.1–25.7]	0.002
% mucinous pattern	31.7 ± 25.2	20.3 ± 21.4	0.17		
Medullary pattern	16 (40.0)	5 (12.5)	0.005 ‡		n.s
% medullary pattern	43.4 ± 32.9	52.4 ± 39.2	0.51		
Micropapillary pattern	4 (10)	4 (10)	1000 †		
% micropapillary pattern	2.3 ± 1.9	26.8 ± 42.3	0.15		
Tumoral necrosis	16 (40)	16 (40)	1000 ‡		
% necrosis	18.1 ± 12.2	10.6 ± 7.9	0.10		
Stromal / malignant cells percentage	16.5 ± 8.3	21.6 ± 12.8	0.02 ¥		n.s
Stromal inflammation			0.82 †		
low	18 (45)	16 (41.0)			
moderate	Z5 (12.5)	6 (15.4)			
high	2 (5.0)	4 (10.3)			
absent	15 (37.5)	13 (33.3)			
Presence of tumor infiltrating lymphocytes	6 (15)	2 (5)	0.26 †		
Tumor invasion front			0.78 ‡		
expansive type	18 (45.0%)	20 (50.0%)			
invasive type	15 (37.5%)	12 (30.0%)			
expansive and invasive	7 (17.5%)	8 (20.0%)			
Serrated adenoma remnant	3 (7.5)	0 (0.0)	0.24 †		
Eosinophilic cytoplasm	19 ± 24.5	4,6 ± 13.1	<0.001 ¥	1.03 [0.99–1.06]	0.08
Discernable nuclei	14,9 ± 15.5	6,1 ± 13.8	0.001 ¥	1.05 [0.99–1.09]	0.06
Vesicular nuclei	15,6 ± 19.2	10.9 ± 16.8	0.14 ¥		
Proeminent nucleolus	18.1 ± 29.4	13 ± 27.7	0.11 ¥		
Nuclear stratification	33.9 ± 34.6	62.7 ± 34.6	<0.001 ¥		NA
Well-ordered brush border	40.6%	62.9%	0.005 ¥		NA
Cytonuclear atypia			0.96 ‡		
low	10 (25.0)	9 (22.5)			
moderate	22 (55.0)	23 (57.5)			
high	8 (20.0)	8 (20.0)			
Perineural invasion	9 (22.5)	9 (22.5)	1000 ‡		
Lymphatic invasion	23 (57.5)	12 (30.0)	0.01 ‡	4.3 [1.3–14.9]	0.02
Vascular invasion	9 (22.5)	10 (25.0)	0.79 ‡		
Budding	8 (20.0)	6 (15.0)	0.556 ‡		
Considered as serrated adenocarcinoma			<0.001 †		NA
Yes	9 (22.5%)	0 (0.0)			
No	21 (52.5)	40 (100.0)			
Potential	10 (25.0)	0 (0.0)			

NA not adopted, *n.s* not significant; ‡: khi-2; †: Fisher test; ¥: Wilcoxon test

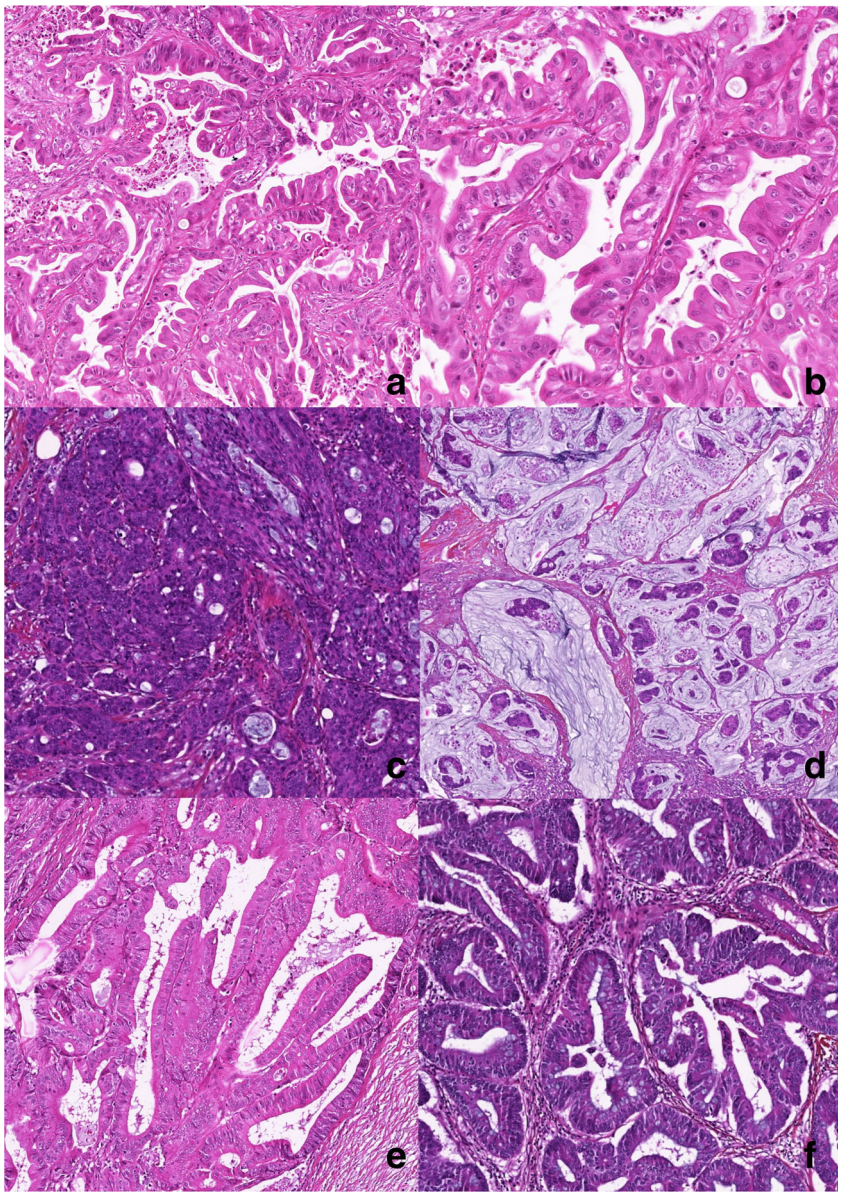

Fig. 2 Histological features of AnnexinA10 + colon adenocarcinomas. **a**, **b** A case of AnnexinA10+ serrated adenocarcinoma (A: ×10 magnification, B: ×20 magnification). **c** A case of AnnexinA10+ medullary adenocarcinoma (× 10 magnification). **d** A case of AnnexinA10 expressing adenocarcinoma with mucinous architecture (× 10 magnification). **e** A case classified as "potential serrated adenocarcinoma" because of the typical cytoplasmic eosinophilia but lack of luminal serration (× 10 magnification). **f** A case of AnnexinA10 negative adenocarcinoma with classical morphology (×10 magnification)

A summary of our results compared with previously published results is available on Supplementary Table 2.

Clinicopathological and Molecular Characteristics of Gremlin1+ Cases

Gremlin1 expression in colonic adenocarcinoma was significantly associated with male gender ($p = 0.002$), younger age ($p = 0.002$), left tumor location ($p = 0.04$), CDX2 expression ($p = 0.004$) and MSS status ($p = 0.04$) in univariate analyses. Gremlin1 expression was not associated with serrated or potentially serrated adenocarcinomas ($p = 0.51$). Details are reported in Table 4. On multivariate analyses, Gremlin1 expression was significantly associated with male gender ($p = 0.008$) and CDX2 expression ($p = 0.02$). Among the 11 cases that co-expressed Gremlin1 and ANXA10 3 (27%) were classified as serrated adenocarcinoma and 2 (18%) as potentially serrated adenocarcinoma. These were not statistically differents than ANXA10+ cases ($p = 0.88$) or Gremlin1 cases ($p = 0.23$).

Survival Analysis

ANXA10 Expression is Correlated with Poor Overall Survival

Survival analyses revealed a tendency of poorer overall survival (OS) of ANXA10+ cases compared with ANXA10- cases ($p = 0.08$; Fig. 3a). Stage specific OS analysis revealed that ANXA10 immunohistochemical expression was an indicator of poorer survival only for UICC stage IV ($p = 0.014$; Fig. 3b). Among stage IV patients from our cohort with

Table 3 Relationships between ANXA10 expression and clinical and molecular characteristics

	Annexin A10		Univariate analysis	Multivariate analysis	
	Positive ($n = 40$) n (%)	Negative ($n = 276$) n (%)	p	OR [IC 95%]	p
Age (Years)			0.79‡		
< 65	13 (32.5)	84 (30.4)			
≥ 65	27 (67.5)	192 (69.6)			
Gender			0.03‡		n.s
Female	24 (60.0)	114 (41.3)			
Male	16 (40.0)	162 (58.7)			
Tumor location			<0.001‡		n.s
Right colon	31 (77.5)	109 (39.5)			
Left colon	9 (22.5)	167 (60.5)			
UICC stage			0.10‡		n.s
I	1 (2.6)	35 (12.8)			
II	12 (30.8)	107 (39.1)			
III	12 (30.8)	68 (24.8)			
IV	14 (35.9)	64 (23.4)			
Differentiation grade			<0.001‡		n.s
1–2	24 (60.0)	241 (87.3)			
3	16 (40.0)	35 (12.7)			
CDX2			0.007 †		n.s
Positive	31 (79.5)	253 (93.7)			
Negative	8 (20.5)	17 (6.3)			
Gremlin1			0.31‡		
Positive	11 (32.3)	104 (41.4)			
Negative	23 (67.7)	147 (58.6)			
KRAS status			0.06‡		
Wild type	24 (82.8)	79 (64.7)			
Mutant	5 (17.2)	43 (35.2)			
BRAF status			<0.001‡	0.03 [0.006–0.16]	<0.001
Wild type	15 (38.5)	250 (92.6)			
Mutant	24 (61.5)	20 (7.4)			
Microsatellite status			<0.001‡		n.s
MSS	20 (51.3)	248 (92.2)			
MSI	19 (48.7)	21 (7.8)			
CIMP status			0.02 ‡		NA
No CIMP	2 (11.8)	21 (46.7)			
CIMP-L	11 (64.7)	21 (46.7)			
CIMP-H	4 (23.5)	3 (6.7)			

NA not adopted, *n.s* not significant; ‡: khi-2; †: Fisher test

available information regarding medical treatment, 55 received 5-fluorouracil-based chemotherapies (LV5FU2, 10; FOLFOX, 16; FOLFIRI, 28; FOLFIRINOX, 1). The most frequently used targeted therapy was the Vascular Endothelial Growth Factor (VEGF) inhibitor bevacizumab (42/85), followed by the Epidermal Growth Factor Receptor (EGFR) inhibitors cetuximab (12/85) and panitumumab (7/85). In patients treated with bevacizumab ($n = 42$), survival analyses revealed a tendency of poorer overall survival (OS) of ANXA10+ cases compared with ANXA10- cases ($p = 0.09$).

There was no significant difference for event-free survival between adenocarcinomas expressing and not expressing ANXA10 whatever the stage or treatment.

Table 4 Relationships between Gremlin1 expression and clinical and molecular characteristics

	Gremlin1		Univariate analysis	Multivariate analysis	
	Positive ($n = 115$) n (%)	Negative ($n = 170$) n (%)	p	OR [IC 95%]	p
Age (Years)			0.03‡	1.6 [0.9–2.7]	0.10
< 65	43 (37.4)	42 (24.7)			
≥ 65	72 (62.6)	128 (75.3)			
Gender			0.002 ‡	2.0 [1.2–3.3]	0.008
Female	37 (32.2)	87 (51.2)			
Male	78 (67.8)	83 (48.8)			
Tumor location			0.04 ‡		n.s
Right colon	41 (35.7)	81 (47.7)			
Left colon	74 (64.3)	89 (52.3)			
UICC stage			0.57 ‡		
I	12 (10.5)	21 (12.5)			
II	43 (37.7)	68 (40.5)			
III	32 (28.1)	35 (20.8)			
IV	27 (23.7)	44 (26.2)			
Differentiation grade			0.47 ‡		
1–2	99 (86.1)	141 (17.1)			
3	16 (13.9)	29 (82.9)			
CDX2			0.004 ‡	4.6 [1.3–16.2]	0.02
Positive	110 (97.4)	146 (87.4)			
Negative	3 (2.7)	21 (12.6)			
Serrated adenocarcinoma			0.51 †		
Yes	3 (11.1)	3 (6.7)			
No	22 (81.5)	35 (77.8)			
Potential	2 (7.4)	7 (15.6)			
KRAS status			0.19 ‡		
Wild type	33 (61.1)	61 (71.8)			
Mutant	21 (38.9)	24 (28.2)			
BRAF status			0.21 ‡		
Wild type	100 (88.5)	137 (83.0)			
Mutant	13 (11.5)	28 (17.0)			
Microsatellite status			0.04 ‡		n.s
MSS	101 (91.0	136 (81.9)			
MSI	10 (9.0)	30 (18.1)			
CIMP status			0.79†		
No CIMP	6 (31.6)	15 (41.7)			
CIMP-L	10 (52.6)	17 (47.2)			
CIMP-H	3 (15.8	4 (11.1)			

NA not adopted, *n.s* not significant; ‡: khi-2; †: Fisher test

Gremlin Expression is Associated with Improved Survival

Survival analyses revealed that Gremlin1 was significantly associated with better OS in UICC stage III colonic adenocarcinoma only ($p = 0.006$; Fig. 4). Gremlin1 expression was not an event-free survival prognosis indicator.

Discussion

Serrated adenocarcinomas were first described by Jass in 1992 as having a close structural and histo-chemical resemblance to hyperplastic polyps with glandular serration [21], and was individualized as CRC subtype in the 2010 and 2019 WHO

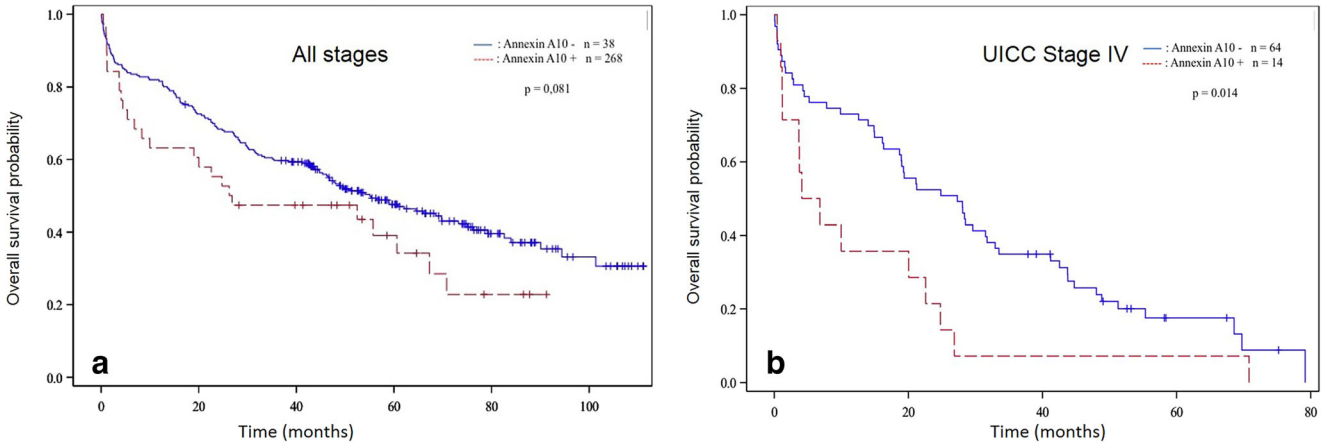

Fig. 3 Survival analysis in colon cancer patients according to Annexin A10 expression status. Kaplan-Meier curves of overall survival probability for AnnexinA10 + (red line) and AnnexinA10 - (blue line) in adenocarcinoma cases for all stages combined (**a**) and in stage IV of UICC (**b**). All p values were calculated using the log rank test

classifications [4]. Since 1992, some studies described the clinical characteristics, the morphology, the molecular profile and the prognosis value of serrated adenocarcinoma [2, 5, 6, 12, 14, 15, 21–29]. These studies described a distinct clinical and molecular profile of SAC: women predominance, higher age, right colonic tumor location, CIMP-H, MSI-Hand *BRAFV600E* mutated status. In current practice, the diagnosis of SAC could be challenging to make because the histological criteria remained imprecise [4, 5]. Some SAC had a distinct molecular profile with CIMP-H and *KRAS* mutation [13, 28]. The prognosis values of this morphological pattern remained controversial [15, 23, 25]. Recently, Annexin A10 and Gremlin1 were identified as a specific marker of the serrated pathway [6, 13, 15, 30]. Precise morphological characteristics of ANXA10+ or Gremlin1+ CRC were not studied. Prognostic value of ANXA10 and Gremlin1 were studied in few studies [12, 13, 15].

In our cohort, ANXA10 expression was observed in 11.6% and Gremlin1 in 40.4% of the cases. Previous studies reported a proportion of 5.8 to 8.8% of ANXA10+ colon adenocarcinoma [6, 12]. The higher percentage observed in our cohort may be either due to the threshold of ANXA10 immunohistochemical positivity used or the origin of the population studied. We used a 1% threshold calculated as a mean of ANXA10 nuclear expression all TMA core. Although, TMA technique is considered as representative of a whole slide immunostaining [6] it remained a selected view of the FFPE block and may underestimate the positivity percentage. In previous studies [6, 12, 13] a 5% threshold was used on TMA but could relate to only one TMA core score [12, 13]. In our cohort, all cases ($n = 13$) with global positivity percentage between 1 and 5% had at least one TMA core that reached the 5% threshold. Thus, if a global approach in previously published studies was used, it may be possible that some cases were reclassified as negative with the 5% threshold but not with the 1%

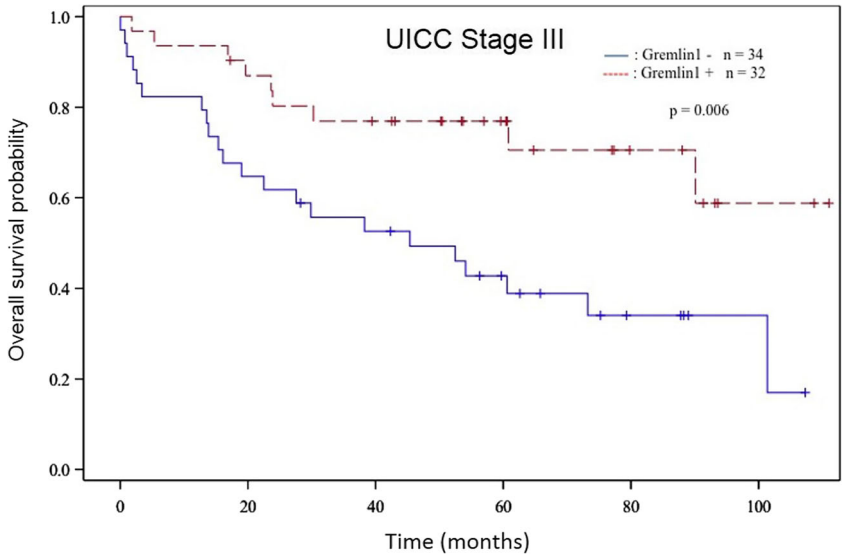

Fig. 4 Survival analysis in colon cancer patients according to Gremlin1 expression. Kaplan-Meier curves of overall survival probability for Gremlin1 + (red line) and Gremlin1 - (blue line) in adenocarcinoma cases in stage III (UICC). All *p* values were calculated using the log rank test

threshold. Another study used a semi-quantitative scoring system and the first positivity scale was 0–25% [14]. Thus, only completely negative cases were considered as negative. All these thresholds reflected that ANXA10 nuclear expression could be very focal in some cases.

The proportion of Gremlin1 expressing adenocarcinoma was not previously reported. ANXA10 and Gremlin1 association were not previously studied. In our study, ANXA10 and Gremlin1 immunohistochemical expression were not statistically associated. Serrated morphology was associated with ANXA10 expression only. Thus, Gremlin1 doesn't seem to be good marker of SAC. Previous association with Gremlin1 expression and serrated or potentially serrated adenocarcinoma subtype may be due to the higher proportion of such adenocarcinoma subtype in the previous study (34.5%) [15] than in our study (5.5%). Moreover, this discrepant findings between the previous study and our results may also be due to the scoring methods used: qualitative (positive/negative) with a 1% threshold in our study and semi-quantitative (intensity x percentage of positivity) with median score comparison in the previous study.

In the present study, the clinico-pathological characteristics associated with ANXA10 expression in colon adenocarcinoma were: female gender, right tumor location, poor differentiation, serrated, mucinous and medullary architecture, cytoplasmic eosinophilia, discernible nuclei, preserved polarity, less well-ordered brush border, less abundant stroma, lymphatic invasion and loss of CDX2 expression. Previous studies reported an association of ANXA10 expression in colon adenocarcinoma with female gender [13], right-sided cancer [6, 12–14], poor differentiation [14], loss of CDX2 expression [14], serrated, mucinous or medullary morphology [6] as in our study. Previous studies also reported an association between ANXA10 expression and lower age at diagnosis [12], advanced TNM stage [12], increased tumor-infiltrating lymphocyte [12], Crohn-like stroma reaction, signet ring cell morphology [14] and lack of necrosis [12, 14]. Age and UICC stage were not associated with ANXA10 expression in another study [6] as in our study. However, in our cohort one third of ANXA10+ case totally lacked the typical serrated morphology. The majority of this non serrated ANXA10+ cases had a predominant medullary architecture. This absence of serrated morphology in a subsequent proportion of ANXA10+ cases had previously been reported [13]. Thus, ANXA10 seems to be a better diagnosis marker of serrated pathway than the typical serrated morphology. Indeed, we found an optimal sensitivity of ANXA10 for the diagnosis of SAC but a lack of specificity.

Although SAC is a WHO entity, some authors do not believe in its existence, partly due to not that easily reproducible diagnostic histological criteria. However, our study, and previous study have shown that SAC had a distinct molecular profile [6, 12, 13, 22, 28] and a poorer prognosis [12, 28, 31] than conventional CCR. Thus, due to this poorer prognosis it seems important to recognize this CCR subtype. ANXA10 may help to diagnose SAC in cases with borderline serrated morphology. ANXA10 immunolabelling, even if focally expressed, is easily assessable. Indeed, the kappa value was strong in our study and in previous study [13].

In the present study ANXA10 was expressed in 47.5% of MSI-H colonic cancer and was also associated with *BRAF* mutation, and CIMP-H status. On the multivariate analysis, ANXA10 expression was independently associated only with *BRAF* mutation. *BRAF* mutation had been previously reported as the early event that promotes the serrated neoplasia pathway [32]. Previous studies also found association between ANXA10 expression and the *BRAF* mutated, MSI-H, CIMP-H molecular profile [12, 14] and also reported a statistical association between ANXA10 expression and wild type *KRAS* status [6, 13, 14]. This association may be due to a mirror effect of the mutual exclusivity of *BRAF* and *KRAS* mutation. In our study, the absence of KRAS mutation was not statistically associated with ANXA10 expression. Indeed, as previously reported some SAC had a distinct molecular profile with *KRAS* mutation, absence of *BRAF* mutation and MSS. These SAC are supposed to derived from traditional serrated adenoma [14, 26]. Thus, according to the consensus molecular subtypes of colorectal cancer [33], ANXA10+ adenocarcinomas would be classified in the CMS1 (MSI Immune) molecular subtype. CMS1 adenocarcinomas had a very poor survival after relapse.

ANXA10 expression was associated with decreased overall survival especially in patients with metastatic disease in our study and in a previous study [12]. In this previous study ANXA10 expression was also associated with poor event-free-survival, especially in metastatic patients. The prognosis impact of ANXA10 immunohistochemical expression in stage IV patients may partly be explained by the association with microsatellite instability. Indeed, stage II-III MSI CRC had a better prognosis than stage II-III MSS CRC. However, stage IV MSI CRC are associated with poor prognosis and chemoresistance, especially to 5FU-based chemotherapy [20]. Thus, role of ANXA10 as an independent prognosis marker of poor overall survival in stage IV colon cancer need to be clarify in the future.

In our series of 346 colon cancers, Gremlin1 expression was significantly associated with male gender, younger age (<65 years), left tumor location, CDX2 expression and MSS status, in univariate analysis. In our cohort, Gremlin1 was an indicator of improved survival in UICC stage III patients. Gremlin1 immunohistochemical expression was previously evaluated on CRC in one study of 148 cases [15]. In this study, Gremlin1 overexpression was associated with low TNM stage, low histological grade, serrated histology and was a stage independent indicator of improved survival. The improved survival observed in this study may be explained by

the association of Gremlin1 overexpression with low TNM stage. However, Gremlin1 expression was not associated with low TNM stage in our study. In our cohort of patient, the better prognosis of stage III colon adenocarcinoma expressing Gremlin1 may partly be explained by its association with CDX2 expression conservation. Indeed, previous studies showed that loss of CDX2 expression was an indicator of poorer prognosis in stage II-III CRC [34, 35].

Conclusion

This study evaluated 2 candidates' markers of the serrated pathway in colon cancer: Annexin10 and Gremlin1. In our case series, Gremlin1 expression was associated neither with serrated morphology nor with the serrated molecular profile. Thus, Gremlin1 doesn't seem to be a marker of the serrated pathway. In the contrary, AnnexinA10 expressing colon adenocarcinomas had the clinico-pathological and molecular features observed in SAC. Indeed, they are more frequently observed in women, are located in the right colon and are often associated with serrated morphology, MSI-H status, CIMP-H status and *BRAF* mutations as previously described in SAC. However, AnnexinA10+ adenocarcinomas may lack the traditional serrated morphological characteristics and are frequently of mucinous or medullary subtype. Colon adenocarcinomas expressing AnnexinA10 are associated with poor overall survival in UICC stage IV.

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Data curation: BMA; CB.
Formal analysis: BMA; AMB; CB.
Funding acquisition: CB-R.
Investigation: BMA; AMB; CF; NB; CB-R.
Methodology: CB.
Project administration: M-DD; CB-R.
Software: CB.
Supervision: OB; M-DD; CB-R.
Validation: OB; RK; M-DD.
Visualization: OB; RK; M-DD.
Roles/Writing - original draft: BMA, CB-R.
Writing - review &editing: CB; OB; RK; AMB; M-DD.

Contributions All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Benjamin Marquet, Aude Marchal Bressenot, Caroline Fichel, Nicole Bouland, Coralie Barbe, Marie-Danièle Diebold and Camille Boulagnon-Rombi. The first draft of the manuscript was written by Benjamin Marquet and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Compliance with Ethical Standards

The study was performed in accordance with the ethical standards. All patients had given their consent for biospecimen use and the study was performed in accordance with the ethical standards laid down in the Declaration of Helsinki. The written consent of patients to the biospecimen use was obtained in all cases. Approval for the study was previously obtained from the local Institutional Review Board and the Tissue Bank Management Board.

Conflict of Interest Authors have no conflict of interest to declare

References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A (2018) Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 68:394–424. https://doi.org/10.3322/caac.21492
2. Mäkinen MJ (2007) Colorectal serrated adenocarcinoma. Histopathology 50:131–150. https://doi.org/10.1111/j.1365-2559.2006.02548.x
3. Mäkinen MJ (2014) Serrated polyps and colorectal cancer risk. Colorectal Cancer 3:77–91. https://doi.org/10.2217/crc.13.84
4. Nagtegaal ID, Odze RD, Klimstra D et al (2019) WHO classification of tumours of the digestive system. In: WHO classification of tumours editorial board, 5th edn. Lyon, pp 157-192
5. Tuppurainen K, Mäkinen JM, Junttila O, Liakka A, Kyllönen AP, Tuominen H, Karttunen TJ, Mäkinen MJ (2005) Morphology and microsatellite instability in sporadic serrated and non-serrated colorectal cancer. J Pathol 207:285–294. https://doi.org/10.1002/path.1850
6. Sajanti SA, Väyrynen JP, Sirniö P, Klintrup K, Mäkelä J, Tuomisto A, Mäkinen MJ (2015) Annexin A10 is a marker for the serrated pathway of colorectal carcinoma. Virchows Arch 466:5–12. https://doi.org/10.1007/s00428-014-1683-6
7. Mussunoor S, Murray G (2008) The role of annexins in tumour development and progression. J Pathol 216:131–140. https://doi.org/10.1002/path.2400
8. Munksgaard PP, Mansilla F, Brems Eskildsen A-S, Fristrup N, Birkenkamp-Demtröder K, Ulhøi BP, Borre M, Agerbæk M, Hermann GG, Ørntoft TF, Dyrskjøt L (2011) Low ANXA10 expression is associated with disease aggressiveness in bladder cancer. Br J Cancer 105:1379–1387. https://doi.org/10.1038/bjc.2011.404
9. Kim J, Kim MA, Jee CD, Jung EJ, Kim WH (2009) Reduced expression and homozygous deletion of annexin A10 in gastric carcinoma. Int J Cancer 125:1842–1850. https://doi.org/10.1002/ijc.24541
10. Liu S-H, Lin C-Y, Peng S-Y, Jeng YM, Pan HW, Lai PL, Liu CL, Hsu HC (2002) Down-regulation of Annexin A10 in hepatocellular carcinoma is associated with vascular invasion, early recurrence, and poor prognosis in synergy with p53 mutation. Am J Pathol 160:1831–1837. https://doi.org/10.1016/S0002-9440(10)61129-7
11. Shimizu T, Kasamatsu A, Yamamoto A, Koike K, Ishige S, Takatori H, Sakamoto Y, Ogawara K, Shiiba M, Tanzawa H, Uzawa K (2012) Annexin A10 in human oral cancer: biomarker for tumoral growth via G1/S transition by targeting MAPK signaling pathways. PLoS One 7:e45510. https://doi.org/10.1371/journal.pone.0045510
12. Bae JM (2015) Annexin A10 expression in colorectal cancers with emphasis on the serrated neoplasia pathway. World J Gastroenterol 21:9749–9757. https://doi.org/10.3748/wjg.v21.i33.9749
13. Kim JH, Rhee Y-Y, Kim K-J, Cho NY, Lee HS, Kang GH (2014) Annexin A10 expression correlates with serrated pathway features in colorectal carcinoma with microsatellite instability. APMIS 122:1187–1195. https://doi.org/10.1111/apm.12284

14. Tsai J-H, Lin Y-L, Cheng Y-C, Chen CC, Lin LI, Tseng LH, Cheng ML, Liau JY, Jeng YM (2015) Aberrant expression of annexin A10 is closely related to gastric phenotype in serrated pathway to colorectal carcinoma. Mod Pathol 28:268–278. https://doi.org/10.1038/modpathol.2014.96
15. Pelli A, Väyrynen JP, Klintrup K, Mäkelä J, Mäkinen MJ, Tuomisto A, Karttunen TJ (2016) Gremlin1 expression associates with serrated pathway and favourable prognosis in colorectal cancer. Histopathology 69:831–838. https://doi.org/10.1111/his.13006
16. Yamasaki Y, Ishigami S, Arigami T, Kita Y, Uchikado Y, Kurahara H, Kijima Y, Maemura K, Natsugoe S (2018) Expression of gremlin1 in gastric cancer and its clinical significance. Med Oncol 35(3): 30. https://doi.org/10.1007/s12032-017-1073-4
17. Sobin LH, Gospodarowicz MK, Wittekind C (2010) TNM classification of malignant tumours. In: Wiley-Blackwell, 7th edn. Chichester, West Sussex, UK, pp 309
18. Wang LM, Kevans D, Mulcahy H, O Sullivan J, Fennelly D, Hyland J, O Donoghue D, Sheahan K (2009) Tumor budding is a strong and reproducible prognostic marker in T3N0 colorectal cancer. Am J Surg Pathol 33:134–141. https://doi.org/10.1097/PAS.0b013e318184cd55
19. Boulagnon C, Dudez O, Beaudoux O, Dalstein V, Kianmanesh R, Bouché O, Diebold MD (2016) BRAFV600E gene mutation in colonic adenocarcinomas. Immunohistochemical detection using tissue microarray and clinicopathologic characteristics: an 86 case series. Appl Immunohistochem Mol Morphol 24:88–96. https://doi.org/10.1097/PAI.0000000000000157
20. Boulagnon-Rombi C, Schneider C, Leandri C, Jeanne A, Grybek V, Bressenot AM, Barbe C, Marquet B, Nasri S, Coquelet C, Fichel C, Bouland N, Bonnomet A, Kianmanesh R, Lebre AS, Bouché O, Diebold MD, Bellon G, Dedieu S (2018) LRP1 expression in colon cancer predicts clinical outcome. Oncotarget 9:8849–8869. https://doi.org/10.18632/oncotarget.24225
21. Jass JR, Smith M (1992) Sialic acid and epithelial differentiation in colorectal polyps and cancer. A morphological, mucin and lectin histochemical study. Pathology 24:233–242. https://doi.org/10.3109/00313029209068874
22. García-Solano J, Pérez-Guillermo M, Conesa-Zamora P, Acosta-Ortega J, Trujillo-Santos J, Cerezuela-Fuentes P, Mäkinen MJ (2010) Clinicopathologic study of 85 colorectal serrated adenocarcinomas: further insights into the full recognition of a new subset of colorectal carcinoma. Hum Pathol 41:1359–1368. https://doi.org/10.1016/j.humpath.2010.04.002
23. Shida Y, Fujimori T, Tanaka H, Fujimori Y, Kimura R, Ueda H, Ichikawa K, Tomita S, Nagata H, Kubota K, Tsubaki M, Kato H, Yao T, Sugai T, Sugihara K, Ohkura Y, Imura J (2012) Clinicopathological features of serrated adenocarcinoma defined by Mäkinen in dukes' B colorectal carcinoma. Pathobiology 79: 169–174. https://doi.org/10.1159/000334837
24. Mäkinen MJ, George SM, Jernvall P et al (2001) Colorectal carcinoma associated with serrated adenoma - prevalence, histological features, and prognosis. J Pathol 193:286–294. https://doi.org/10.1002/1096-9896(2000)9999:9999<::AID-PATH800>3.0.CO;2-2
25. Lee C-T, Huang Y-C, Hung L-Y, Chow NH, Su PF, Ho CL, Tsai HW, Chen YL, Lin SC, Lin BW, Lin PC, Lee JC (2017) Serrated adenocarcinoma morphology in colorectal mucinous adenocarcinoma is associated with improved patient survival. Oncotarget 8: 35165–35175. https://doi.org/10.18632/oncotarget.16815
26. Stefanius K, Ylitalo L, Tuomisto A, Kuivila R, Kantola T, Sirniö P, Karttunen TJ, Mäkinen MJ (2011) Frequent mutations of KRAS in addition to BRAF in colorectal serrated adenocarcinoma: KRAS and BRAF in serrated adenocarcinoma. Histopathology 58:679–692. https://doi.org/10.1111/j.1365-2559.2011.03821.x
27. Conesa-Zamora P, García-Solano J, García-García F, Turpin MC, Trujillo-Santos J, Torres-Moreno D, Oviedo-Ramírez I, Carbonell-Muñoz R, Muñoz-Delgado E, Rodriguez-Braun E, Conesa A, Pérez-Guillermo M (2013) Expression profiling shows differential molecular pathways and provides potential new diagnostic biomarkers for colorectal serrated adenocarcinoma. Int J Cancer 132: 297–307. https://doi.org/10.1002/ijc.27674
28. Kim JH, Bae JM, Cho N-Y, Kang GH (2016) Distinct features between *MLH1*-methylated and unmethylated colorectal carcinomas with the CpG island methylator phenotype: implications in the serrated neoplasia pathway. Oncotarget 7:14095–14111. https://doi.org/10.18632/oncotarget.7374
29. García-Solano J, Conesa-Zamora P, Carbonell P, Trujillo-Santos J, Torres-Moreno D D, Pagán-Gómez I, Rodríguez-Braun E, Pérez-Guillermo M (2012) Colorectal serrated adenocarcinoma shows a different profile of oncogene mutations, MSI status and DNA repair protein expression compared to conventional and sporadic MSI-H carcinomas. Int J Cancer 131:1790–1799. https://doi.org/10.1002/ijc.27454
30. Gonzalo DH, Lai KK, Shadrach B, Goldblum JR, Bennett AE, Downs-Kelly E, Liu X, Henricks W, Patil DT, Carver P, Na J, Gopalan B, Rybicki L, Pai RK (2013) Gene expression profiling of serrated polyps identifies annexin A10 as a marker of a sessile serrated adenoma/polyp. J Pathol 230:420–429. https://doi.org/10.1002/path.4200
31. De Sousa E Melo F, Wang X, Jansen M et al (2013) Poor-prognosis colon cancer is defined by a molecularly distinct subtype and develops from serrated precursor lesions. Nat Med 19:614–618. https://doi.org/10.1038/nm.3174
32. Rad R, Cadiñanos J, Rad L, Varela I, Strong A, Kriegl L, Constantino-Casas F, Eser S, Hieber M, Seidler B, Price S, Fraga MF, Calvanese V, Hoffman G, Ponstingl H, Schneider G, Yusa K, Grove C, Schmid RM, Wang W, Vassiliou G, Kirchner T, McDermott U, Liu P, Saur D, Bradley A (2013) A genetic progression model of BrafV600E-induced intestinal tumorigenesis reveals targets for therapeutic intervention. Cancer Cell 24:15–29. https://doi.org/10.1016/j.ccr.2013.05.014
33. Guinney J, Dienstmann R, Wang X, de Reyniès A, Schlicker A, Soneson C, Marisa L, Roepman P, Nyamundanda G, Angelino P, Bot BM, Morris JS, Simon IM, Gerster S, Fessler E, de Sousa E Melo F, Missiaglia E, Ramay H, Barras D, Homicsko K, Maru D, Manyam GC, Broom B, Boige V, Perez-Villamil B, Laderas T, Salazar R, Gray JW, Hanahan D, Tabernero J, Bernards R, Friend SH, Laurent-Puig P, Medema JP, Sadanandam A, Wessels L, Delorenzi M, Kopetz S, Vermeulen L, Tejpar S (2015) The consensus molecular subtypes of colorectal cancer. Nat Med 21:1350–1356. https://doi.org/10.1038/nm.3967
34. Dalerba P, Sahoo D, Paik S, Guo X, Yothers G, Song N, Wilcox-Fogel N, Forgó E, Rajendran PS, Miranda SP, Hisamori S, Hutchison J, Kalisky T, Qian D, Wolmark N, Fisher GA, van de Rijn M, Clarke MF (2016) CDX2 as a prognostic biomarker in stage II and stage III colon cancer. N Engl J Med 374:211–222. https://doi.org/10.1056/NEJMoa1506597
35. Tomasello G, Barni S, Turati L, Ghidini M, Pezzica E, Passalacqua R, Petrelli F (2018) Association of CDX2 expression with survival in early colorectal cancer: a systematic review and meta-analysis. Clin Colorectal Cancer 17:97–103. https://doi.org/10.1016/j.clcc.2018.02.001