

The serrated neoplasia pathway of colorectal tumors: Identification of MUC5AC hypomethylation as an early marker of polyps with malignant potential

Florence Renaud, Christophe Mariette, Audrey Vincent, Agnès Wacrenier, Vincent Maunoury, Julie Leclerc, Lucie Coppin, Michel Crépin, Isabelle van Seuningen, Emmanuelle Leteurtre, et al.

▶ To cite this version:

Florence Renaud, Christophe Mariette, Audrey Vincent, Agnès Wacrenier, Vincent Maunoury, et al.. The serrated neoplasia pathway of colorectal tumors: Identification of MUC5AC hypomethylation as an early marker of polyps with malignant potential. International Journal of Cancer, 2016, 138, pp.1472-1481. 10.1002/ijc.29891 . hal-02905823

HAL Id: hal-02905823 https://hal.science/hal-02905823

Submitted on 2 Oct 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers. L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

International Journal of Cancer



The serrated neoplasia pathway of colorectal tumours: identification of MUC5AC hypomethylation as an early marker of high-risk polyps

Journal:	International Journal of Cancer			
Manuscript ID:	IJC-15-1083			
Wiley - Manuscript type:	Early Detection and Diagnosis			
Date Submitted by the Author:	22-May-2015			
Complete List of Authors:	Renaud, Florence; Institut de Pathology, Mariette, Christophe; Lille University Hospital, Digestive Surgery Vincent, Audrey; Inserm UMRS 1172, Team 'Mucins, Epithelial Differentiation and carcinogenesis' Wacrenier, Agnes; Lille University Hospital, Pathology Institute Maunoury, Vincent; Lille University Hospital, Gastroenterology Leclerc, Julie; Lille University Hospital, Molecular Oncology and Genetics, Biochemistry and Molecular Biology Institute Coppin, Lucie; Lille University Hospital, crépin, michel; Lille university hospital, Van Seuningen, Isabelle; Inserm UMRS 1172, Team 'Mucins, Epithelial Differentiation and carcinogenesis' Leteurtre, Emmanuelle; Lille University Hospital, Molecular Oncology and Genetics, Biochemistry and Molecular Biology Institute			
Key Words:	hyperplastic polyp, sessile serrated adenoma, colonic cancer, mucin, methylation			
	·			

SCHOLARONE[™] Manuscripts

Renaud et al.

The serrated neoplasia pathway of colorectal tumours: identification of *MUC5AC* hypomethylation as an early marker of high-risk polyps

Short title: MUC5AC hypomethylation in high-risk colorectal serrated polyps

F Renaud^{1,2,3}, C Mariette^{1,3,4}, A Vincent^{1,3,5}, A Wacrenier², V Maunoury⁶, J Leclerc^{1,3,7}, L Coppin^{1,3,7}, M Crépin⁷, I Van Seuningen^{1,3,5}, E Leteurtre^{1,2,3}, MP Buisine^{1,3,7}

¹ Inserm, UMR-S1172, Team 'Mucins, Epithelial Differentiation and Carcinogenesis', Jean-Pierre Aubert Research Center, Lille, France

² Pathology Institute, Biology Pathology Center, Lille University Hospital, Lille, France

³ North of France Lille 2 University, Lille, France

⁴ Department of Digestive Surgery, Claude Huriez Hospital, Lille University Hospital, Lille, France

⁵ Lille University Hospital, Lille, France

⁶ Department of Gastroenterology, Claude Huriez Hospital, Lille University Hospital, Lille, France

⁷ Department of Molecular Oncology and Genetics, Biochemistry and Molecular Biology Institute, Biology Pathology Center, Lille University Hospital, Lille, France

Key words: hyperplastic polyp, sessile serrated adenoma, colonic cancer, mucin, methylation.

Abbreviations: CA, conventional adenoma; CIMP, CpG island methylation phenotype; CIMP-H, CIMP-high; CIMP-L, CIMP-low; CRC, colorectal carcinoma; GCHP, goblet cellrich hyperplastic polyp; MSI, microsatellite instability; MSS, microsatellite stable; MVHP,

microvesicular hyperplastic polyp; SSA, sessile serrated adenoma; SSA/D, sessile serrated adenoma with dysplasia; TSA, traditional serrated adenoma.

Grant sponsors: SIRIC ONCOLille; Lille University Hospital; French Society of Pathology.

Correspondence to: Marie-Pierre Buisine, Inserm UMR-S1172, Team 'Mucins, Epithelial Differentiation and Carcinogenesis', Jean-Pierre Aubert Research Center, 59045 Lille cedex, France. Tel.: 33-3-20-29-88-50, Fax: 33-3-20-53-85-62, E-mail: marie-pierre.buisine@inserm.fr

Appropriate article category: Early detection and diagnosis

ABSTRACT

The serrated neoplasia pathway accounts for 20 to 30% of colorectal cancers (CRC), which are characterised by extensive methylation (CpG Island Methylation Phenotype, CIMP), frequent BRAF mutation and high microsatellite instability (MSI). We recently identified MUC5AC mucin gene hypomethylation as a specific marker of MSI CRC. The early identification of preneoplastic lesions among serrated polyps is currently challenging. Here we performed a detailed pathological and molecular analysis of a large series of colorectal serrated polyps and evaluated the usefulness of mucin genes MUC2 and MUC5AC to differentiate serrated polyps and to identify high-risk lesions. A series of 330 colorectal polyps including 218 serrated polyps (42 goblet cell-rich hyperplastic polyps (GCHP), 68 microvesicular hyperplastic polyps (MVHP), 100 sessile serrated adenoma (SSA), 8 traditional serrated adenoma (TSA)) and 112 non-serrated polyps was analysed for BRAF/KRAS mutations, MSI, CIMP, MLH1 and MGMT methylation, and MUC2 and *MUC5AC* expression and methylation. We show that *MUC5AC* hypomethylation is an early event in the serrated neoplasia pathway, and specifically detects MVHP and SSA, arguing for a filiation between MVHP, SSA, and CIMP-H/MSI CRC, whereas GCHP and TSA arise from a distinct pathway. Moreover, MUC5AC hypomethylation specifically identified serrated lesions with *BRAF* mutation, CIMP-H or MSI, suggesting that it may be useful to identify serrated neoplasia pathway-related precursor lesions. Our data suggest that MVHP and SSA should benefit from the same therapeutic and surveillance strategies.

What's new?

The identification of high-risk colorectal serrated polyps is challenging since benign hyperplastic polyps and sessile serrated adenoma may share the same morphology and since carcinogenesis in this pathway is faster than in the conventional adenoma-carcinoma pathway. We identified MUC5AC hypomethylation as a new early marker of serrated neoplasia pathway, specifically detecting CIMP-H/MSI CRC precursors MVHP and SSA, suggesting they should benefit from the same surveillance for accurate management of patients.

Renaud et al.

INTRODUCTION

Most colorectal cancers (CRC) develop through a conventional adenoma (CA)-carcinoma sequence. However, 20 to 30% of CRC develop through the 'serrated neoplasia pathway', named as for the serrated appearance of crypt in the precursor polyps.¹⁻³

Serrated lesions represent a heterogeneous group of polyps, divided into hyperplastic polyps (HP), sessile serrated adenomas (SSA) and traditional serrated adenomas (TSA) by the latest World Health Organisation (WHO) classification of tumors of the digestive system (fourth edition 2010).⁴ However a diagnostic 'grey-zone' exists between HP and SSA, with a wide inter-observer variability due to the lack of consensus diagnostic criteria.⁵⁻⁹ In addition, contradictory guidelines were given for the diagnosis of serrated polyps by the WHO and then by an expert panel,¹⁰ resulting in some confusion among pathologists.⁹ Yet, it is important to distinguish SSA from HP, since the former are associated with a malignant potential whereas the later are commonly considered to be benign. Furthermore, neoplastic progression through this pathway has been reported to be faster than within the conventional adenoma-carcinoma pathway. Underdiagnosis of serrated neoplasia pathway precursors in pathologic practice may result in inadequate surveillance and thus contribute to interval carcinomas.⁹

SSA were underdiagnosed for many years due to their resemblance to HP and it currently remains unanswered whether SSA arise *de novo* or derive from pre-existing HP.^{11,12}

Molecular characterization has improved our understanding of serrated polyps and associated-risk of malignant progression. SSA are mainly observed in the proximal colon and are associated with frequent *BRAF* mutation and high CpG island methylation phenotype (CIMP-H), suggesting that SSA are precursors of sporadic CIMP-H CRC, most of which display high microsatellite instability (MSI) through epigenetic inactivation of *MLH1*.^{2,11,13} TSA seem to be less strongly associated with the serrated neoplasia pathway, but they are

associated with frequent *KRAS* or *BRAF* mutations, low CIMP (CIMP-L) and are supposed to be precursors of CIMP-L microsatellite stable (MSS) CRC.

Moreover, serrated pathway-related polyps and CIMP-H/MSI CRC have similar phenotype, including frequent mucinous pattern with increased expression of the intestinal mucin MUC2 and aberrant expression of the gastric mucin MUC5AC.^{14,15} We recently showed that abnormal expression of MUC2 and MUC5AC in CIMP-H/MSI CRC was closely related to altered methylation of their promoter.¹⁶ Interestingly, *MUC5AC* demethylation was specific to MSI CRC, suggesting that it may serve as a specific marker of the serrated neoplasia pathway.

In this study, 330 polyps including 218 serrated polyps were selected in order to (1) further characterise molecular features of serrated polyps and (2) evaluate the potential interest of mucin genes *MUC2* and *MUC5AC* to discriminate serrated polyps and to identify high-risk lesions. We demonstrate that *MUC5AC* hypomethylation is an early and specific marker of preneoplastic lesions in the serrated neoplasia pathway of the colorectum.



Renaud et al.

MATERIAL AND METHODS

Patients and tissues

Specimens of colorectal serrated lesions and conventional adenoma were retrieved from the archives of the Department of Pathology of the Lille University Hospital. All cases were collected from patients who underwent endoscopic mucosal resection, polypectomy or colectomy at Lille University Hospital from 2009 to 2013. Polyps were selected on the basis that they fulfilled the diagnostic criteria, were well oriented, and had sufficient available tissue for both immunohistochemical and molecular analyses. Clinical and endoscopic data were available for all patients. Clinical information retrieved included sex and age, colonic localisation (classified as proximal or distal relative to the splenic flexure) and size of the polyp. Informed consent was obtained from all patients. Approval of this study was obtained from the Institutional Review Board of Lille University Hospital.

Histological analysis

All specimens were reviewed by three experienced gastrointestinal pathologists (EL, AW, FR) blinded to the clinical and molecular information. Serrated lesions (HP, SSA and TSA) were classified on the basis of the criteria from the latest WHO classification.⁴ Representative serrated polyps included in this study are shown in Figure 1. Mixed polyps or cases with hyperplastic polyposis were excluded from this study. HP were defined as serrated polyps with simple tubular architecture composed of serrated crypts in the upper half of the lesion, narrow and straight crypts at the base. HP were subdivided into two groups: microvesicular HP (MVHP) and goblet cell-rich HP (GCHP) defined as per previously published criteria.⁴ MVHP were characterized by elongated and relatively straight crypts, with serration that are visible mainly near the luminal end of the crypts, epithelial cells with microvesicular (small

droplet) mucin and absence of cytologic dysplasia (Figure 1A). GCHP were characterized by elongated and slightly serpiginous crypts mostly composed of mucin-rich goblet cells (Figure 1B). SSA were defined as serrated polyps with basal crypt dilatation, crypt branching, horizontal extension of crypts at the base, prominent serrations, mitoses in the upper half of crypts and dystrophic goblet cells (Figure 1C). To classify a polyp as SSA, there should be at least two or three contiguous SSA-type crypts. Dysplasia was defined by the presence of narrow elongated hyperchromatic nuclei, nuclei stratification and basophilic cytoplasm (Figure 1D). TSA were defined as serrated polyps composed of a uniform population of cells showing abundant eosinophilic cytoplasm, nuclear elongation, ectopic crypt formations and nuclear hyperchromasia with a tubular or villous architecture and mild crypt or surface slit-like serration (Figures 1E and 1F).

In total, 330 polyps from 330 patients were included in the study. They contained 218 serrated polyps with 110 HP (68 MVHP, 42 GCHP), 100 SSA (41 SSA with dysplasia), 8 TSA (3 with dysplasia). Non-serrated adenomas (112 CA including 17 tubular, 18 villous, 77 tubulovillous) diagnosed during the same period were also included as controls.

Immunohistochemistry

Immunohistochemical studies were carried out on 4-µm-thick formalin-fixed, paraffinembedded whole tissue sections using antibodies against mucins MUC2 and MUC5AC as previously described.¹⁷ The average percentage of stained tumour cells and intensity of staining were calculated using a semiquantitative histological scoring method (Hscore).¹⁸ Hscore above or equal the threshold of 20 was considered as positive expression.

DNA methylation analysis

The promoter methylation status of 6 genes commonly used to define CIMP status (*CDKN2A* (*p16*), *RUNX3*, *CACNA1G*, *SOCS1*, *NEUROG1*, and *IGF2*), DNA repair genes *MLH1* and *MGMT*, and mucin genes *MUC2* and *MUC5AC* was determined by quantitative bisulfite pyrosequencing assays as previously described.¹⁶ Amplification of *MGMT* and *MLH1* was performed using the PyroMark kits (Qiagen). The percentage of methylation for a given gene was calculated as the mean methylation levels of all CpG sites analysed. Tumours with a methylation level above or equal to the threshold of 10% were considered as methylation-positive. Tumours with 1 or 2 methylated markers and tumours with no methylated marker were considered as CIMP-L and CIMP-negative, respectively. Hypomethylation was defined by a methylation level below the thresholds of 70% for *MUC5AC* and 60% for *MUC2*, as previously described.¹⁶

MSI and mutation analysis

MSI was determined using a six-marker panel as previously described.¹⁹ Mutations in codons 12 and 13 of *KRAS* and in codon 600 of *BRAF* were identified by pyrosequencing as previously described.¹⁶

Statistical analysis

SPSS version 19.0 software (SPSS, Chicago, IL) and GraphPad Prism version 6.02 were used for statistical analyses. Discrete variables were compared using the Chi square test or the Fischer exact test as appropriate. Continuous variables were compared using the Mann– Whitney U test. A 2-tailed probability value (P) was calculated. A P value < 0.05 was considered statistically significant. CIMP-L and CIMP-negative tumours were grouped vsCIMP-H tumours for statistical analyses.

RESULTS

Clinicopathological characteristics of the cohort

Table 1 summarises the clinical and endoscopic findings of the patients and their polyps.

Among the 330 polyps, 153 were from women and 177 from men, with overall median age of 65 years (range, 32 to 88). Patients with TSA were significantly older than patients with other polyps (P = 0.04). There were no significant different sex ratio between the subtypes of polyps or between patients with serrated polyps (HP, SSA, TSA) and those with CA.

Overall, the median size of polyps was 10 mm (range, 3 to 35 mm) (Table 1). Among serrated polyps, HP were significantly smaller (median, 4.9 mm) than SSA (median, 9 mm), SSA/D (median, 10) and TSA (median, 20 mm) (P < 0.0001, P < 0.0001 and P < 0.0001, respectively).

In terms of tumour site, 44% were proximal and 56% were distal (Table 1). Compared to CA, serrated polyps were more frequently found in the proximal colon (P < 0.001). Among serrated polyps, 53% of MVHP, 69% of SSA, and 71% of SSA/D were proximal, whereas only 5% of GCHP and 25% of TSA were proximal.

Mutational and methylation characteristics of polyp subtypes

Activating mutations in *BRAF* or *KRAS*, MSI and abnormal methylation of a number of genes are common events in preneoplastic colorectal lesions. All cases were successfully analysed for these molecular events. Molecular alterations within the different polyp types are shown in Figure 2.

Compared to CA, serrated polyps displayed higher frequency of *BRAF* V600E mutation (21.6% vs 1.8%), MSI (17.9% vs 0%) and CIMP-H (24.8% vs 5.4%) and lower frequency of

International Journal of Cancer

Renaud et al.

KRAS mutations (11.5% *vs* 33.9%). *BRAF* and *KRAS* mutations were mutually exclusive in all types of polyps.

Among GCHP, 0% displayed *BRAF* mutation, whereas 47.6% displayed *KRAS* mutation, and 0% was MSI. Only 2.4% were CIMP-H, but 11.9% were CIMP-L and 9.5% displayed abnormal methylation of *MGMT*. Conversely, among MVHP, 33.8% displayed *BRAF* mutation, 0% *KRAS* mutation, 8.8% were MSI, and 13.2% were CIMP-H. *BRAF* mutations, MSI and CIMP were more common in proximal MVHP compared to distal MVHP (47.2% vs 18.7%, P = 0.02, 16.7% vs 0%, P = 0.03, and 25.0% vs 0%, P = 0.0025, respectively).

In SSA and SSA/D, *BRAF* mutations were significantly more frequent than *KRAS* mutations (22.0% vs 2.0%), 33.0% were MSI with methylation of *MLH1*. 41.0% of SSA were CIMP-H and 8.0% displayed methylation of *MGMT*. *BRAF* mutations, MSI and CIMP were more common in proximal SSA compared to distal SSA (28.0% vs 9.7%, P = 0.06, 43.0% vs 9.0%, P = 0.001, and 59.5% vs 4.0%, P < 0.0001, respectively).

In TSA, *KRAS* mutations were detected in 37.5% of cases, *BRAF* in 25.0% and all were MSS. TSA were essentially CIMP-negative (50.0%) or CIMP-L (37.5%), and were particularly prone to *MGMT* methylation (37.5%). Notably, CIMP-L, detected in 7.3% of all polyps, was significantly associated with *MGMT* methylation (P < 0.0001).

Taken together, our findings argue for a relationship between MVHP and SSA, GCHP and TSA being apart. Moreover, comparison of methylation patterns between the different sub-groups showed an increase in the number of methylation-positive CIMP-target genes and in the methylation levels from MVHP to SSA to SSA/D (Figure 2 and Figure 3A).

Mucin expression in subtypes of polyps

Immunohistochemistry for the intestinal mucin MUC2 and the gastric mucin MUC5AC was performed and analysed in all polyps. Frequencies and representative images of expression

within the different types of polyps are shown in Figure 4 and Additional supporting information.

MUC2 and MUC5AC expressions were more frequent in serrated polyps than in CA (95% vs 63%, P < 0.0001 and 96% vs 59%, P < 0.0001, respectively). Moreover, in HP (GCHP and MVHP), SSA, SSA/D and TSA, MUC2 and MUC5AC staining intensity was generally strong to moderate and homogeneously distributed along the epithelium, whereas it was more heterogeneous in CA.

Mucin gene methylation in subtypes of polyps and association with clinicopathologic features

Methylation of *MUC2* and *MUC5AC* mucin genes was analysed in all polyps. Results are shown in Figure 2 and Figure 3B.

MUC2 mean methylation level was 59.2% in serrated polyps and 63.8% in CA. *MUC2* hypomethylation was more frequent in serrated polyps than in CA (36.7% *vs* 18.8%, P < 0.0001). Among serrated polyps, *MUC2* hypomethylation was detected in 50.0% of GCHP, 20.6% of MVHP, 44.1% of SSA, 43.9% of SSA/D and 12.5% of TSA. In GCHP, *MUC2* hypomethylation was significantly associated with *KRAS* mutation (P = 0.006). In MVHP, *MUC2* hypomethylation was associated with proximal location (P = 0.001) and CIMP (P = 0.002). In SSA, *MUC2* hypomethylation was associated with proximal location (P = 0.001), *BRAF* mutation (P < 0.0001), MSI (P < 0.0001), and CIMP (P < 0.0001).

MUC5AC mean methylation level was 82.5% in serrated polyps and 92.3% in CA. *MUC5AC* hypomethylation was detected in 18.8% of serrated polyps and none of the CA, indicating that *MUC5AC* hypomethylation is specific of the serrated pathway (P < 0.0001). Furthermore, in serrated polyps, *MUC5AC* hypomethylation was detected in 8.8% MVHP, 25.4% SSA, and 43.9% SSA/D, but in none of GCHP or TSA. In MVHP, *MUC5AC*

Renaud et al.

hypomethylation was associated with proximal location (P = 0.011), *BRAF* mutation (P = 0.017), MSI (P < 0.0001), *MLH1* methylation (P < 0.0001), and CIMP-H (P < 0.0001). In SSA (with or without dysplasia), *MUC5AC* hypomethylation was associated with proximal location (P < 0.0001), *BRAF* mutation (P < 0.0001), MSI (P < 0.0001), *MLH1* methylation (P < 0.0001), *MLH1* methylati

MUC5AC hypomethylation is predictive of high-risk serrated lesions

We further evaluated the performances of *MUC5AC* hypomethylation for the identification of lesions harbouring molecular alterations, which should thus be considered as at high risk of progression to carcinoma.

MUC5AC hypomethylation was detected in 52.7% (39/74) of serrated polyps harbouring *BRAF* mutation, CIMP-H, or MSI and in none (0/144) of the other serrated polyps, which corresponds to a positive predictive value of 100% and a negative predictive value of 81.4% for the identification of high-risk serrated lesions.

By comparison, MUC2 hypomethylation was detected in 52.7% (39/74) of serrated polyps harbouring *BRAF* mutation, CIMP-H or MSI, but also in 27.8% (40/144) of the others, which corresponds to a positive predictive value of 49.4% and a negative predictive value of 75.9% for the identification of high-risk serrated lesions.

DISCUSSION

Despite the improved recognition of the clinicopathological significance of serrated lesions of the colorectum, some questions remain to be resolved, such as the differential diagnosis of serrated polyp subtypes and especially the identification of those which require close surveillance. In this study, we performed a detailed pathological and molecular analysis of secreted mucin genes *MUC2* and *MUC5AC*. We show that *MUC5AC* hypomethylation is specific of high-risk serrated polyps, suggesting that *MUC5AC* hypomethylation may serve as a marker to identify serrated pathway neoplasia-related precursors.

Clinicopathological and molecular features were characterized in our series of polyps. Clinicopathological data were in agreement with previous reports, ²⁰⁻²³ confirming that most SSA and MVHP are proximal, whereas most TSA and GCHP are distal. In line with recent reports, ^{11,13,24,25} we found that SSA, especially proximal SSA/D, harboured frequent *BRAF* mutation, were often CIMP-H and MSI due to *MLH1* methylation. Moreover, although the number of TSA in our series was small, we confirmed that they may display *KRAS* or *BRAF* mutations, ²⁶⁻²⁸ were MSS, and often CIMP-L with methylation of *MGMT*. Regarding HP, MVHP differed from GCHP in that they harboured frequent *BRAF* mutation, some of them being CIMP-H and MSI. Conversely, GCHP showed exclusively *KRAS* mutations, were MSS, CIMP-negative or CIMP-L, but prone to *MGMT* methylation.

A common feature of serrated pathway-related polyps and CIMP-H/MSI CRC is frequent mucin-rich/mucinous pattern with increased expression of secreted mucins. We analysed the expression of the secreted mucins MUC2 and MUC5AC in our series of polyps. According to prior studies,^{29,30} increased expression of the intestinal mucin MUC2 and aberrant expression of the gastric mucin MUC5AC was observed in most serrated polyps, especially in MVHP

International Journal of Cancer

Renaud et al.

and SSA. Accumulating evidence indicates that serrated pathway-associated colorectal tumours may display aberrant gastric-type differentiation. However, MUC5AC expression was also observed in CA, although it was less frequent and weaker than in serrated polyps. Our findings, in line with recent ones,³⁰ demonstrate that gastric-type expression is not exclusive to serrated pathway-associated lesions.

Based on our recent data showing that abnormal expression of MUC2 and MUC5AC in CRC was closely related to demethylation of their promoters,¹⁶ we determined the methylation status of *MUC2* and *MUC5AC* in our series of polyps. Hypomethylation of *MUC2* and *MUC5AC* was detected in a proportion of serrated lesions including HP, indicating that the demethylation process is an early event in polyp development. This mechanism may contribute to increased production of mucus and to acquisition of their particular mucin-rich phenotype.

It has been suggested that SSA may originate from certain HP. The frequent finding of MVHP-like areas within large SSA and identification of excessive methylation and *BRAF* mutations in both MVHP and SSA are evidence supporting that SSA may represent 'advanced' forms of MVHP, but the relationship between these two entities has not been clearly established.³¹⁻³³

A key finding of this study was that *MUC5AC* hypomethylation was restricted to MVHP and SSA, suggesting it may be useful to discriminate GCHP from MVHP and SSA. Moreover, there was a gradual increase in the frequency of *MUC5AC* hypomethylation from MVHP to SSA and SSA/D suggesting that SSA arise from MVHP. Interestingly, our recent data in CRC showed that *MUC5AC* hypomethylation was specific to MSI/CIMP-H CRC.¹⁶ Altogether, our data argue for a progression of MVHP to SSA, SSA/D, and then to MSI/CIMP-H CRC (Figure 5). Some authors have questioned the utility of distinguishing

MVHP and GCHP.^{10,34} Our data reinforce the concept that MVHP and GCHP are distinct morphological but also biological entities, highlighting a true relevance to diagnose MVHP and to precise the HP subtype in pathological reports.

We further evaluated the performances of *MUC5AC* hypomethylation in identifying highrisk lesions. *MUC5AC* hypomethylation accurately identified only serrated lesions with *BRAF* mutation, CIMP-H or MSI, with a positive predictive value of 100% and a negative predictive value of 81.4%, validating the utility of *MUC5AC* hypomethylation to assess the malignant risk of precursor lesions.

Furthermore, our data suggest that *MUC5AC* demethylation may play a role in the serrated pathway to colorectal cancer. Understanding the mechanisms leading to *MUC5AC* demethylation and subsequent aberrant expression of the gastric-type MUC5AC mucin could help to elucidate whether it is directly involved in the progression of tumours through this pathway.

Until recently, information regarding TSA was very scarce, probably due to their rarity, accounting for less than 1% of all colorectal polyps.⁴ Recent studies on large series of TSA have enriched our knowledge regarding their clinicopathological and molecular features.^{27,28} Whereas TSA are morphologically fairly similar, they are characterized by molecular heterogeneity, showing *BRAF* or *KRAS* mutation and variable CIMP, suggesting at least two pathways of neoplastic progression. Frequent identification of SSA-type lesions in proximal and *BRAF* mutant TSA and frequent association with MVHP led to the hypothesis that some TSA develop from SSA.²⁸ However, no *MUC5AC* hypomethylation was detected in TSA in our series, especially in the two proximal TSA-with *BRAF* V600E mutation. Although the number of TSA was small in our series, our data suggest a pathway distinct from the MVHP-SSA-CIMP-H CRC pathway, as previously suggested by Tsai *et al.*²⁷ Our hypothesis is

International Journal of Cancer

Renaud et al.

supported by recent findings obtained *in vivo* in a *BRAF*-mutant mouse model, which showed that the resulting mice developed serrated polyps which progressed to TSA, but not to SSA.³⁵ Conversely, we observed similarities between TSA with *KRAS* mutation and some GCHP that were CIMP-L with *MGMT* methylation, suggesting that some TSA may develop from GCHP and progress to conventional adenoma and CIMP-negative or CIMP-L MSS CRC (Figure 5).²⁷

Our data have major implication at the clinical level. The identification of high-risk serrated polyp is currently challenging, but is essential to guide adequate cancer screening and prevention care, especially the neoplastic progression within this pathway has been reported to be faster than within the classical adenoma-carcinoma pathway.^{36,37}

In current clinical practice, whereas the diagnosis of SSA is followed by the same colonoscopic surveillance as for patient with CA,³⁴ the diagnosis of HP including MVHP does not lead to a follow-up. Identification of MVHP as precursor lesions of SSA could impact medical practice.

In conclusion, this study further confirms the importance to accurately diagnose serrated lesions. We have shown that *MUC5AC* hypomethylation may be useful to distinguish serrated polyps and to identify high-risk lesions. Moreover, our findings suggest that some MVHP may progress to SSA, SSA/D, and CIMP-H/MSI CRC. In this respect, MVHP and SSA should benefit from the same therapeutic and surveillance strategies.

Acknowledgements:

The authors thank B. Duchêne, RM Siminsky and MH Gevaert for excellent technical assistance.

References

 Snover DC, Jass JR, Fenoglio-Preiser C, Batts KP. Serrated polyps of the large intestine: a morphologic and molecular review of an evolving concept. *Am J Clin Pathol* 2005;**124**:380-91.

2. Jass JR. Classification of colorectal cancer based on correlation of clinical, morphological and molecular features. *Histopathology* 2007;**50**:112-130.

3. Bettington M, Walker N, Clouston A, Brown I, Leggett B, Whitehall V. The serrated pathway to colorectal carcinoma: current concepts and challenges. *Histopathology* 2013;**62**:367-86.

4. Snover D, Ahnen DJ, Burt RW, et al. Serrated polyps of the colon and rectum and serrated polyposis. In: Carneiro F, Flejou JF, eds. WHO classification of Tumours of the Digestive System. 4th ed. Lyon: IARC Press;2010.

5. Farris AB, Misdraji J, Srivastava A, Muzikansky A, Deshpande V, Lauwers GY, Mino-Kenudson M. Sessile serrated adenoma: challenging discrimination from other serrated colonic polyps. *Am J Surg Pathol* 2008;**32**:30-5.

Torlakovic EE, Gomez JD, Driman DK, Parfitt JR, Wang C, Benerjee T, Snover DC.
 Sessile serrated adenoma (SSA) vs. traditional serrated adenoma (TSA). *Am J Surg Pathol* 2008;**32**:21-9. Erratum in: *Am J Surg Pathol* 2008;**32**:491.

 Renaud et al.

7. Hetzel JT, Huang CS, Coukos JA, Omstead K, Cerda SR, Yang S, O'Brien MJ, Farraye FA. Variation in the detection of serrated polyps in an average risk colorectal cancer screening cohort. *Am J Gastroenterol* 2010;**105**:2656-64.

8. Ensari A1, Bilezikçi B, Carneiro F, Doğusoy GB, Driessen A, Dursun A, Flejou JF, Geboes K, de Hertogh G, Jouret-Mourin A, Langner C, Nagtegaal ID, *et al.* Serrated polyps of the colon: how reproducible is their classification? *Virchows Arch* 2012;**461**:495-504.

9. Bettington M, Walker N, Rosty C, Brown I, Clouston A, Wockner L, Whitehall V, Leggett
B. Critical appraisal of the diagnosis of the sessile serrated adenoma. *Am J Surg Pathol* 2014;**38**:158-66.

10. Rex DK, Ahnen DJ, Baron JA, Batts KP, Burke CA, Burt RW, Goldblum JR, Guillem JG, Kahi CJ, Kalady MF, O'Brien MJ, Odze RD, *et al.* Serrated lesions of the colorectum: review and recommendations from an expert panel. *Am J Gastroenterol* 2012;**107**:1315-29.

11. Spring KJ, Zhao ZZ, Karamatic R, Walsh MD, Whitehall VL, Pike T, Simms LA, Young J, James M, Montgomery GW, Appleyard M, Hewett D, *et al.* High prevalence of sessile serrated adenomas with BRAF mutations: a prospective study of patients undergoing colonoscopy. *Gastroenterology* 2006;**131**:1400-7.

12. Batts KP. The pathology of serrated colorectal neoplasia: practical answers for common questions. *Modern Path* 2015;**28**:S80-S87.

13. Kambara T, Simms LA, Whitehall VL, Spring KJ, Wynter CV, Walsh MD, Barker MA, Arnold S, McGivern A, Matsubara N, Tanaka N, Higuchi T, *et al.* BRAF mutation is associated with DNA methylation in serrated polyps and cancers of the colorectum. *Gut* 2004;**53**:1137-44.

14. Bara J, Forgue-Lafitte E and Buisine MP. Expression of gastric MUC5AC mucin during colon carcinogenesis. In: Immunohistochemistry and in situ hybridization of human carcinomas. M.A Hayat Eds 2005: 167-182.

15. Gibson JA, Hahn HP, Shahsafaei A, Odze RD. MUC expression in hyperplastic and serrated colonic polyps: lack of specificity of MUC6. *Am J Surg Pathol* 2011;**35**:742-9.

16. Renaud F, Vincent A, Mariette C, Crépin M, Stechly L, Truant S, Copin MC, Porchet N, Leteurtre E, Van Seuningen I, Buisine MP. MUC5AC hypomethylation is a predictor of microsatellite instability independently of clinical factors associated with colorectal cancer. *Int J Cancer* 2015;**136**:2811-21.

17. Buisine MP, Desreumaux P, Leteurtre E, Copin MC, Colombel JF, Porchet N, Aubert JP. Mucin gene expression in intestinal epithelial cells in Crohn's disease. *Gut* 2001;**49**:544-51.

Cappia S, Righi L, Mirabelli D, Ceppi P, Bacillo E, Ardissone F, Molinaro L, Scagliotti GV, Papotti M. Prognostic role of osteopontin expression in malignant pleural mesothelioma.
 Am J Clin Pathol 2008;130:58-64.

International Journal of Cancer

Renaud et al.

19. Pagin A, Zerimech F, Leclerc J, Wacrenier A, Lejeune S, Descarpentries C, Escande F, Porchet N, Buisine MP. Evaluation of a new panel of six mononucleotide repeat markers for the detection of DNA mismatch repair-deficient tumours. *Br J Cancer* 2013;**108**:2079-87.

20. Fujita K, Yamamoto H, Matsumoto T, Hirahashi M, Gushima M, Kishimoto J, Nishiyama K, Taguchi T, Yao T, Oda Y. Sessile serrated adenoma with early neoplastic progression: a clinicopathologic and molecular study. *Am J Surg Pathol* 2011;**35**:295-304.

21. Patil DT, Shadrach BL, Rybicki LA, Leach BH, Pai RK. Proximal colon cancers and the serrated pathway: a systematic analysis of precursor histology and BRAF mutation status. *Mod Pathol* 2012;**25**:1423-31.

22. Salaria SN, Streppel MM, Lee LA, Iacobuzio-Donahue CA, Montgomery EA. Sessile serrated adenomas: high-risk lesions? *Hum Pathol* 2012;**43**:1808-14.

23. Bettington M, Walker N, Clouston A, Brown I, Leggett B, Whitehall V. The serrated pathway to colorectal carcinoma: current concepts and challenges. *Histopathology* 2013;**62**:367-86.

24. Jass JR, Baker K, Zlobec I, Higuchi T, Barker M, Buchanan D, Young J. Advanced colorectal polyps with the molecular and morphological features of serrated polyps and adenomas: concept of a 'fusion' pathway to colorectal cancer. *Histopathology* 2006;**49**:121-31.

John Wiley & Sons, Inc.

25. Kim YH, Kakar S, Cun L, Deng G, Kim YS. Distinct CpG island methylation profiles and BRAF mutation status in serrated and adenomatous colorectal polyps. *Int J Cancer* 2008;**123**:2587-93.

26. Fu B, Yachida S, Morgan R, Zhong Y, Montgomery EA, Iacobuzio-Donahue CA.
Clinicopathologic and genetic characterization of traditional serrated adenomas of the colon. *Am J Clin Pathol* 2012;**138**:356-66.

27 Tsai JH, Liau JY, Lin YL, Lin LI, Cheng YC, Cheng ML, Jeng YM. Traditional serrated adenoma has two pathways of neoplastic progression that are distinct from the sessile serrated pathway of colorectal carcinogenesis. *Mod Pathol* 2014;**27**:1375-85.

28. Bettington ML, Walker NI, Rosty C, Brown IS, Clouston AD, McKeone DM, Pearson SA, Klein K, Leggett BA, Whitehall VL. A clinicopathological and molecular analysis of 200 traditional serrated adenomas. *Mod Pathol* 2015;**28**:414-27.

29. Fujita K, Hirahashi M, Yamamoto H, Matsumoto T, Gushima M, Oda Y, Kishimoto J, Yao T, Iida M, Tsuneyoshi M. Mucin core protein expression in serrated polyps of the large intestine. *Virchows Arch* 2010;**457**:443-9.

30. Kim JH, Kim KJ, Rhee YY, Bae JM, Cho NY, Lee HS, Kang GH. Gastric-type expression signature in serrated pathway-associated colorectal tumours. *Hum Pathol* 2015;**46**:643-56.

Renaud et al.

31. Rosty C, Buchanan DD, Walsh MD, Pearson SA, Pavluk E, Walters RJ, Clendenning M, Spring KJ, Jenkins MA, Win AK, Hopper JL, Sweet K, *et al.* Phenotype and polyp landscape in serrated polyposis syndrome: a series of 100 patients from genetics clinics. *Am J Surg Pathol* 2012;**36**:876-82.

32. Rosty C, Parry S, Young JP. Serrated polyposis: an enigmatic model of colorectal cancer predisposition. Patholog *Res Int* 2011:157073. doi: 10.4061/2011/157073.

33. Kwon HJ, Cho NY, Chang MS, Kim YS, Kang GH. Intermediate serrated polyp as an intermediate lesion of hyperplastic polyp and sessile serrated polyp/adenoma in terms of morphological and molecular features. Hum Pathol. 2014;**45**:1759-65.

34. Lieberman DA, Rex DK, Winawer SJ, Giardiello FM, Johnson DA, Levin TR; United States Multi-Society Task Force on Colorectal Cancer. Guidelines for colonoscopy surveillance after screening and polypectomy: a consensus update by the US Multi-Society Task Force on Colorectal Cancer. *Gastroenterology* 2012;**143**:844-57.

35. Rad R, Cadinanos J, Rad L, Varela I, Strong A, Kriegl L, Constantino-Casas F, Eser S, Hieber M, Seidler B, Price S, Fraga MF, *et al.* A genetic progression model of BRAFV600E-induced intestinal tumourigenesis reveals targets for therapeutic intervention. *Cancer Cell* 2013;**24**:15-29.

36. Lazarus R, Junttila OE, Karttunen TJ, Mäkinen MJ. The risk of metachronous neoplasia in patients with serrated adenoma. *Am J Clin Pathol* 2005;**123**:349-59.

Renaud et al.

37. Goldstein NS. Serrated pathway and APC (conventional)-type colorectal polyps: molecular-morphologic correlations, genetic pathways, and implications for classification. *Am J Clin Pathol* 2006;**125**:146-53.

Table 1. Clinical and endoscopic findings

Polyp type	HP	SSA	SSA/D	TSA	CA
No. of cases	110	59	41	8	112
Male:Female ratio	60:50	35:24	18:23	3:5	61:51
Median age (y) (range)	65.5 (34-88)	61 (32-83)	63 (43-82)	71 (60-85)	65 (45-82)
Median size (mm) (range)	4.9 (3-8)	9 (5-20)	10 (5-30)	20 (15-30)	15 (8-35)
Proximal:Distal colon	38:72	41:18	29:12	2:6	34:78

HP, hyperplastic polyp; SSA, sessile serrated adenoma; SSA/D, sessile serrated adenoma with dysplasia; TSA, traditional serrated adenoma; CA, conventional adenoma

Figure Legends.

Figure 1. Representative serrated colorectal polyps included in the study.

A. Microvesicular hyperplastic polyp (MVHP) located in the proximal colon, 20 x magnification. Relatively straight crypts are mostly composed of microvesicular epithelial cells.

B. Goblet cell-rich hyperplastic polyp (GCHP) located in the distal colon, with slightly serpiginous crypts and numerous mucin-rich goblet cells, 40 x magnification.

C. Sessile serrated adenoma (SSA) located in the proximal colon, 20 x magnification. Note the serrations that extend to the crypt base, basal crypt dilatation, horizontal growth and absence of cytologic dysplasia.

D. SSA with low-grade dysplasia (SSA/D), defined by enlarged hyperchromatic nuclei, nuclear stratification and loss of polarity, 20 x magnification.

E. Traditional serrated adenoma (TSA), with protuberant villous architecture, 5 x magnification.

F. TSA with low-grade dysplasia, 10 x magnification. Note slit-like serrations (arrows), epithelial cells with abundant brightly eosinophilic cytoplasm with central nuclei, ectopic crypts formations.

Figure 2. Schematic representation of anatomic, mutational and methylation characteristics of polyps.

Horizontal rows represent individual polyp. Each column shows the tumour site and the presence or absence of mutation (*KRAS*, *BRAF*), microsatellite instability (MSI), abnormal promoter methylation (*MLH1*, *MGMT*, CIMP-related genes, *MUC2*, *MUC5AC*), CpG island methylator phenotype (CIMP). Dark grey squares indicate proximal site, presence of

International Journal of Cancer

Renaud et al.

mutation, MSI, hypermethylation of *MLH1*, *MGMT*, CIMP markers and high CIMP; light grey squares indicate low CIMP; white squares indicate distal site, absence of mutation, MSI, methylation for *MLH1*, *MGMT*, CIMP markers and hypomethylation of *MUC2* or *MUC5AC* (see materials and methods for details). GCHP, goblet cell-rich hyperplastic polyps; MVHP, microvesicular hyperplastic polyp; SSA, sessile serrated adenoma; SSA/D, sessile serrated adenoma; CA, conventional adenoma.

Figure 3. Methylation profiles in colorectal polyps.

These graphs show individual methylation levels for CIMP markers (A) and mucin genes (B) in the different polyp subtypes: goblet cell-rich hyperplastic polyp (GCHP), microvesicular hyperplastic polyp (MVHP), sessile serrated adenoma (SSA), sessile serrated adenoma with dysplasia (SSA/D), traditional serrated adenoma (TSA) and conventional adenoma (CA). Each circle represents an individual specimen; the horizontal bars represent means values. Indicated comparisons correspond to GCHP *vs* MVHP, and to each serrated polyp subtype *vs* CA (Fisher exact test). Only significant *P*-values are shown. Note the restriction of *MUC5AC* hypomethylation to MVHP, SSA and SSA/D and the increase in methylation levels from MVHP to SSA and to SSA/D for CIMP markers and mucin genes.

Figure 4. Mucin immunohistochemical expression in colorectal polyps.

Representative photomicrographs of immunohistochemistry for MUC2 and MUC5AC in colorectal polyps. Note the positivity of MUC2 and MUC5AC in hyperplastic polyps (HP), sessile serrated adenomas (SSA) and in traditional serrated adenomas (TSA). The staining was homogeneous along the crypt, with a moderate or high intensity. The staining was more heterogeneous in conventional adenoma (CA) in terms of frequency, intensity and distribution.

Figure 5. Schematic representation of putative pathways to colorectal cancer.

GCHP, goblet cell-rich hyperplastic polyp; MVHP, microvesicular hyperplastic polyp; SSA, sessile serrated adenoma; SSA/D, sessile serrated adenoma with dysplasia; TSA, traditional serrated adenoma; CA, conventional adenoma; CIN, chromosomal instability; CIMP, CpG island methylator phenotype; CIMP-H, CIMP-high; CIMP-L, CIMP-low; MSI, microsatellite instability; MSS, microsatellite stable.

ISS, microsac....







Β.







