Reappraisal of the so-called ‘villous tumours’ of the rectosigmoid, based on histological, immunohistochemical and genotypic features

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Abstract

Background: Villous tumours of the rectosigmoid are historically defined as broad-based lesions associated with secretory diarrhoea.

Objective: This study aimed to perform a reappraisal of these tumours, on the basis of newly introduced histological, immunohistochemical and molecular parameters.

Methods: For this study, 22 villous tumours, diagnosed by endoscopic criteria (19 Paris 0-IIa, three Paris 0-Is), were evaluated according to WHO classification. Microsatellite instability status, *KRAS* and *BRAF* mutations, *MGMT* status of villous tumours and associated invasive carcinoma were determined.

Results: The 22 villous tumours fell into four groups: 1) nine villous adenomas, 2) six tubulovillous adenomas, 3) three filiform traditional serrated adenomas, and 4) four traditional serrated adenomas with conventional dysplasia. Filiform serrated adenomas displayed a distinctive endoscopic protruding pattern (Paris 0-Is). Villous adenomas were strongly associated with secretory diarrhoea. All the villous tumours were microsatellite stable. Five tumours exhibited *MGMT* abnormalities. *KRAS* mutations were frequent in villous adenomas, whereas *BRAF* mutations were essentially detected in serrated lesions. Invasive carcinomas ($n=7$) maintained the histopathological and molecular imprint of the prior villous tumour.

Conclusion: The rectosigmoid villous tumours are histologically and molecularly heterogeneous, including serrated neoplasias. Endoscopic and clinical findings are predictive of the histopathological diagnosis of some of these distinct entities.

Keywords

Rectal neoplasms, villous tumour, endoscopy, microsatellite instability, serrated adenoma

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Introduction

The so-called ‘villous tumour’ of the rectosigmoid was first described by Quénu and Landel in 1899.¹ These authors described very large broad-based rectal tumours associated with secretory diarrhoea. Later, Bensaude succinctly puts it: “the polyp bleeds, while the villous tumour weeps.” Thereafter, this important clinical feature was described as the McKittrick-Wheelock syndrome.² Currently, the diagnosis of ‘villous tumour’ is proposed by clinicians on the endoscopic pattern of protuberant soft mass or friable sessile mass with a ‘shaggy’ surface or ‘fingerlike’ processes. Histologically, villous tumours are defined by the extent of the villous component. After the publication of the 1976 World Health Organization (WHO) classification

of colorectal tumours,³ the villous tumours were simply included with other polyps in the broad category of adenomas, characterized by dysplasia. In this setting, villous tumours lost their clinicopathological specificity. Recently however, the apparent homogeneity of the epithelial polyps was challenged on the basis of

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histological and molecular criteria.^{4–6} In the last version of the WHO classification of colorectal tumours,^{7,8} this has resulted in the recognition of two separate entities, i.e. adenomas with the so-called conventional dysplasia, including villous, tubulovillous and tubulous adenomas, and serrated polyps/adenomas, with or without serrated dysplasia, and/or additional conventional dysplasia. However, despite these changes, this classification did not recognize the villous tumours as a separate entity. Interestingly, a villous pattern can be displayed by some serrated adenomas, sometimes termed filiform serrated adenomas.⁹ To date, however, there has been no attempt to reappraise the villous tumours initially characterized by Quénu and Landel, on the basis of this newly described WHO classification.

Materials and methods

Patient samples

All tumours were diagnosed as ‘villous tumour’ at endoscopic examination and were resected surgically or by endoscopic mucosal resection between 2000 and 2010 at Nantes University Hospital. Fujinon high-resolution endoscopes (EC450, EC530 or EC590

series) were mostly used during this period of time. Indigo-carmin chromoendoscopy was also routinely performed. In contrast, we did not use immersion or magnification techniques on these lesions. The tumours were selected from a retrospective review of endoscopic reports archived in institutional files, based on the following criteria: location in the rectosigmoid; and endoscopic appearance of protuberant mass of size ≥ 1 cm, with a broad-based and a shaggy or velvety structure that covers the whole surface area. Each report was reviewed by a senior endoscopist (EC) to ensure accurate description of the lesions. In total, 22 villous tumours from 22 patients were selected, and classified endoscopically according to the Paris endoscopic classification of neoplastic lesions.¹⁰ Clinical data were available for the entire series (Table 1). This study was conducted in accordance with ethical guidelines of French public health laws.

Histological evaluation of villous tumours

Villous tumours, as defined by endoscopic criteria, were reviewed by two pathologists (LDD, JFM), and were classified according to the current WHO classification of colorectal tumours.^{7,8} Villous tumours were

Table 1. Clinical, endoscopic and pathological features of the villous tumours

	All villous tumours (%)	VA (%)	TVA (%)	FSA (%)	TSAD (%)
Patients	22	9	6	3	4
Mean age	73	78	71	73	65
Gender (male: female)	10: 12	4: 5	2: 4	1: 2	3: 1
Clinical presentation					
McKittrick-Wheelock syndrome	7 (32)	5 (56)	1 (17)	0 (0)	1 (25)
Rectal bleeding	6 (27)	0 (0)	3 (50)	0 (0)	3 (75)
Screening colonoscopy	9 (41)	4 (44)	2 (33)	3 (100)	0 (0)
Localization					
Sigmoid	4 (18)	1 (11)	0 (0)	2 (67)	1 (25)
Rectum	18 (82)	8 (89)	6 (100)	1 (33)	3 (75)
Endoscopic features					
Large broad-based villous lesion (Paris 0-IIa)	19 (86)	9 (100)	6 (100)	1 (33)	3 (75)
Protrusive sessile villous polyp (Paris 0-Is)	3 (14)	0 (0)	0 (0)	2 (67)	1 (25)
Mean size cm (range)	5.2 (1–14)	7.5 (2.5–14)	4.5 (2–6.5)	1.8 (1–3.5)	3.8 (1.5– 5.5)
Vienna classification					
3	4 (18)	0 (0)	3 (50)	1 (33)	0 (0)
4–1	11 (50)	6 (67)	2 (33)	1 (33)	2 (50)
4–4	1 (5)	1 (11)	0 (0)	0 (0)	0 (0)
5	4 (18)	2 (22)	1 (17)	0 (0)	1 (25)
Invasion beyond submucosa	2 (9)	0 (0)	0 (0)	1 (33)	1 (25)

VA: villous adenoma; TVA: tubulovillous adenoma; FSA: filiform serrated adenoma; TSAD: traditional serrated adenoma with conventional dysplasia.

classified based on the following criteria: percentage of villi, and type of epithelial dysplasia covering the villi. Villous tumours were classified as villous adenoma when the villous component represented more than 80% of the whole tumour surface, and tubulovillous adenoma when the villous component ranged between 60% and 80% of the tumour surface. Epithelial dysplasia was classified as conventional dysplasia, or as 'eosinophilic' or 'serrated' dysplasia. Conventional dysplasia was defined by narrow elongated hyperchromatic nuclei and basophilic cytoplasm; 'eosinophilic' or 'serrated' dysplasia was defined by cuboidal cells with eosinophilic cytoplasm and less packed vesicular nuclei with prominent nucleoli. Carcinomatous components of villous tumours were classified based on the current WHO classification.⁷ In addition, the Vienna classification of gastrointestinal epithelial neoplasia¹¹ was used to evaluate the stage of the lesions.

Immunohistochemical evaluation of villous tumours

One representative block from each tumour was processed for immunohistochemical staining. All immunostains were performed on 4 µm-thick, formalin-fixed, paraffin-embedded tissue sections. The standard avidin-biotin complex technique was used on deparaffinized sections. Immunostaining with primary antibodies described in Table 2 was analysed by two observers (LDD, JFM). Nuclear immunostaining with MLH1, MSH2, MSH6, and MGMT was considered negative when no neoplastic epithelial cell exhibited staining.

DNA extraction

A desired area of the polyp (adenoma or carcinoma) was selected on formalin-fixed paraffin-embedded sections stained with hematoxylin and eosin. The selected tissue was manually macrodissected from 10 µm-thick sections. DNA was purified, then washed and concentrated with Amicon Ultra Centrifugal Filters® (Merck

KGaA, Darmstadt, Germany), according to the manufacturer's instructions.

KRAS and BRAF mutation analysis, microsatellite analysis

PCR amplification and direct sequencing determined mutations of *KRAS* codon 12 and 13. PCR amplification was performed using COLD-PCR method (co-amplification at lower denaturation temperature PCR).¹² The primer set covered codon 12 and 13 of the *KRAS* gene. PCR products were directly sequenced using ABI PRISM 3130XL DNA sequencer® (Applied Biosystems), as previously described.¹³

BRAF V600E mutations were detected using quantitative allele-specific PCR, as designed and described previously.¹⁴ PCR primers for the hotspot region of the *BRAF* gene on exon 15 (T1799A) were used. *BRAF* V600E mutations were determined using Rotorgene 2000® (Corbett Research).

The microsatellite instability (MSI) status was determined by PCR to amplify five mononucleotide markers, BAT25, BAT26, NR21, NR22 and NR24, as previously described.¹⁵ Briefly, the five mononucleotide repeats were coamplified in a single pentaplex PCR reaction. PCR products were analysed on an ABI PRISM 3100 capillary automated DNA sequencer. Genscan software (Genotyper 2.1, Applera, France) was used to calculate the size of each fluorescent PCR product.

Methylation analysis

hMLH1 and *MGMT* promoter methylation was determined using MethyLight method.¹⁶ Tumour DNA was subjected to sodium bisulfite conversion using Epitect Bisulfite kit (Qiagen®), according to the manufacturer's recommendations. The *COL2A1* control reaction was used to normalize for bisulfite-converted input DNA. The samples were classified as positive for *hMLH1* or *MGMT* promoter methylation when the amount of methylated DNA (PMR) ≥ 10.

Table 2. Summary of immunohistochemical markers

Marker	Clone	Dilution	Company
MUC2	NCL-MUC-2	1:300	Novocastra, Newcastle, UK
MUC5AC	anti-MUC5AC ab	1:100	gift of Dr J. Bara, INSERM U482, hôpital St Antoine, Paris
MLH1	554072	1:100	Biosciences, Palo Alto, CA USA
MSH2	NA26	1:20	Calbiochem, San Diego, CA, USA
MSH6	81374	prediluted	Zymed Laboratories Invitrogen, San Francisco, USA
MGMT	MT3.1	1:200	Merck Millipore, Billerica, MA

Results

Clinical and endoscopic characteristics of the villous tumours

Clinical features of the villous tumours. Among 7310 recto-sigmoid polyps resected over 10 years in our institution, 22 tumours diagnosed as 'villous tumours' on endoscopic examination were selected.

The clinical features of the 22 villous tumours are summarized in Table 1. None of the patients gave personal or close family history of invasive carcinoma. Seven patients (35%) presented with McKittrick–Wheelock syndrome, manifested by secretory diarrhoea with fluid and electrolyte depletion.

Endoscopic features of the villous tumours. Of the villous tumours, 19 were identified as large, broad-based sessile

villous lesions (predominantly Paris 0–IIa) and three tumours appeared as smaller (1–3.5 cm), more protrusive sessile villous lesions (Paris 0–Is) (Figure 1(a), (c)). All the tumours had a shaggy or combined shaggy and finely lobular pattern. Twenty tumours had soft consistency while two other tumours were fixed, suggestive of invasive lesions. Size of the villous tumours ranged from 1 cm to 14 cm (Table 1).

Microscopic features of the villous tumours

The villous tumours, as defined by endoscopic criteria, fell into four categories, based on the WHO classification of colorectal tumours,^{7,8} i.e. villous adenomas, tubulovillous adenomas, filiform serrated adenomas, and traditional serrated adenomas with conventional dysplasia. In fact, of the 22 villous tumours, nine

Figure 1. Endoscopic and histological aspects of villous tumours. (a) Broad-based large sessile tumour, predominantly Paris 0–IIa in the rectum, corresponding to (c) villous adenoma. (b) Protrusive lesion in the rectosigmoid. Described as 'villous tumour' in the initial endoscopic report, classified as Paris 0–Is, this lesion corresponded in fact to (d) filiform serrated adenoma.

(41%) matched the precise definition of villous adenomas (Figure 1(b)), i.e. their architecture exhibited more than 80% villi. These tumours contained numerous immature foveolar-like mucin-secreting cells and a few goblet cells. They displayed both low-grade and high-grade conventional dysplasia.

Six tumours (27%) corresponded to tubulovillous adenomas, composed of mixed villous and tubular structures, and showing from 60–80% villous architecture. They exhibited both low-grade and high-grade conventional dysplasia. Mainly basophilic columnar cells, with some interspersed goblet cells, lined these tumours. Immature foveolar-like mucin-secreting cells were present in only one tumour.

Three tumours (14%) corresponded to traditional serrated adenomas with villous architecture (Figure 1(d)), also described as ‘filiform serrated adenomas’.⁹ They showed long filiform projections lined by a serrated epithelium, and some ectopic crypt formations. The neoplastic epithelium was composed mainly of penicillar eosinophilic cells, with some oligomucin immature goblet cells. These tumours exhibited serrated dysplasia, characterized by cuboidal cells with eosinophilic cytoplasm and slightly packed vesicular nuclei with prominent nucleoli.

Four tumours (18%) exhibited concomitantly the patterns of villous adenomas and serrated filiform adenomas. These tumours were originally diagnosed as mixed hyperplastic and adenomatous polyps, by Longacre and Fenoglio,^{4,8} and fulfilled the criteria of traditional serrated adenoma with conventional dysplasia described by Fu et al.¹⁷ (Figure 2(d)). These lesions exhibited both low-grade and high-grade conventional dysplasia and serrated patterns. Table 1 shows the stage of the lesions, according to the Vienna classification.¹¹

Mucin gene expression

Almost all villous tumours (19/22, 86%), regardless of their histological type, demonstrated staining for the intestinal MUC2 mucin. MUC2 expression was restricted to mature goblet cells and oligomucin immature goblet cells. Interestingly, the expression of the gastric MUC5AC mucin was observed in immature foveolar-like mucin-secreting cells of most of the villous adenomas (8/9, 89%). It was also observed in goblet cells of two filiform serrated adenomas and the three traditional serrated adenomas with conventional dysplasia. Only one tubulovillous adenoma showed MUC5AC expression (Table 3, Figure 2).

Figure 2. Mucin immunophenotype of a (a) villous adenoma and a (d) traditional serrated adenoma with conventional dysplasia. (b, e) MUC2 perivacuolar expression in oligomucin immature goblet cells. (c, f) MUC5AC vacuolar expression in goblet cells and in foveolar immature mucin-secreting cells. * Ectopic crypt formations.

Table 3. Immunohistochemical and molecular features of the villous tumours and their carcinomatous transformation

	All villous tumours <i>n</i> = 22 (%)	VA <i>n</i> = 9 (%)	TVA <i>n</i> = 6 (%)	FSA <i>n</i> = 3 (%)	TSAD <i>n</i> = 4 (%)
MUC2	19 (86)	8 (89)	5 (83)	3 (100)	3 (75)
MUC5AC	14 (64)	8 (89)	1 (17)	2 (66)	3 (75)
Microsatellite instability status					
MSS	22 (100)	9 (100)	6 (100)	3 (100)	4 (100)
MSI	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
MGMT methylation	5 (23)	2 (22)	2 (33)	1 (33)	0 (0)
MGMT loss of immunostaining	2 (9)	1 (11)	0 (0)	1 (33)	0 (0)
KRAS mutation	12 (55)	7 (78)	2 (33)	0 (0)	3 (75)
BRAF V600E mutation	5 (23)	0 (0)	1 (17)	3 (100)	1 (25)
Carcinomatous transformation	7 (32)	3 (33) ¹	1 (17) ¹	1 (33) ²	2 (50) ³

¹Adenocarcinomas, not otherwise specified, pT1N0M0 (derived from two VA and one TVA) or pTisN0M0 (derived from one VA)

²poorly differentiated serrated adenocarcinoma, pT3N1M1a

³mucinous adenocarcinoma, pT2N0M0 and serrated adenocarcinoma with mucinous component, pT1N0M0

VA: villous adenoma; TVA: tubulovillous adenoma; FSA: filiform serrated adenoma; TSAD: traditional serrated adenoma with conventional dysplasia

Microsatellite instability status

Expression of MMR proteins MLH1, MSH2 and MSH6 as assessed by immunohistochemistry was present in all villous tumours. Accordingly, the tumours were found microsatellite stable on molecular analysis. Finally, promoter methylation of *hMLH1* gene was never observed (Table 3).

MGMT promoter methylation status and gene expression

MGMT promoter methylation was observed in five tumours: two villous adenomas, two tubulovillous adenomas, and one filiform serrated adenoma. Of these five tumours, only two, one villous adenoma and one filiform serrated adenoma, showed loss of MGMT staining (Table 3).

KRAS and BRAF mutation analysis

KRAS or BRAF gene mutations were found in a high proportion of tumours (17/22, 77%). KRAS mutations occurred more frequently in villous adenomas (7/9, 78%) and traditional serrated adenomas with conventional dysplasia (3/4, 75%). BRAF V600E mutation was present in all three filiform serrated adenomas (3/3, 100%) and in one traditional serrated adenoma with conventional dysplasia (1/4, 25%). KRAS and BRAF mutations were mutually exclusive (Table 3).

Clinico-pathological correlations

Of the seven patients who presented with McKittrick–Wheelock syndrome, five displayed villous adenomas, one displayed a tubulovillous adenoma with high percentage of villi and numerous immature foveolar-like mucin-secreting cells, and one displayed a traditional serrated adenoma with conventional dysplasia. All these tumours showed strong MUC5AC expression. The other patients, asymptomatic or with rectal bleeding, displayed lesions falling into the four aforementioned categories, with weak or moderate MUC5AC expression.

Lesions identified endoscopically as large broad-based lesions (Paris 0–IIa), corresponded to villous adenomas (9/9, 100%), tubulovillous adenomas (6/6, 100%), or traditional serrated adenomas with conventional dysplasia (3/4, 75%). Three tumours appeared as small (1–3 cm), protrusive sessile villous lesions (Paris 0–Is). All of them corresponded to serrated lesions, i.e. filiform serrated adenomas (2/3, 66%) and traditional serrated adenoma with conventional dysplasia (1/4, 25%) (Table 1).

Malignant transformation of the villous tumours

An invasive component was frequently observed in villous tumours (7/22, 32%). Three villous adenomas and one tubulovillous adenoma were associated with an adenocarcinoma, with mucosal or submucosal infiltration. One filiform serrated adenoma was associated

with a poorly differentiated serrated adenocarcinoma that invaded the subserosa, and spread into lymph nodes and the liver. Two out of four traditional serrated adenomas with conventional dysplasia led to mucinous adenocarcinomas, one of them displaying a serrated component (Table 3). All the invasive carcinomas maintained the same immunohistochemical and molecular profile as their parental villous tumours. In fact, like the parental tumours, they were microsatellite stable and maintained *MLH1*, *MSH2*, *MSH6* expression. In addition, no methylation of *MLH1* and *MGMT* promoter was detected. Finally, the same *KRAS* or *BRAF* mutations were detected in invasive carcinomas and their respective parent tumour. *BRAF* mutation was associated with two of the villous tumours with invasive component and serrated pattern.

Discussion

On the basis of histological, immunohistochemical and molecular findings, we can draw several conclusions regarding the relations between the gross morphology / endoscopic features of the villous tumours and their associated biological characteristics.

Endoscopic predictions

Our results show that the apparent endoscopic homogeneity of the villous tumours translates into a histological and molecular heterogeneity, which is endoscopically relevant. In our series, the villous tumours were endoscopically described as broad-based tumours with a villous pattern. According to the WHO classification, these tumours fell into four groups, namely villous adenomas, tubulovillous adenomas, traditional serrated adenomas with conventional dysplasia and the entity called filiform serrated adenomas. None of these histopathologically defined groups was recognizable on endoscopic examination, with the exception of adenomas with a serrated component. In fact, they were more protruding and smaller than the other villous tumours. Here we show that some villous tumours belong to the so-called serrated pathway of neoplasia, while they do not meet the usual endoscopic features of serrated tumours. Retrospectively, these tumours were frequently classified in category Paris 0–Is rather than Paris 0–IIa.

The McKittrick–Wheelock syndrome is associated with a specific form of villous tumours

In our series, most tumours of the group of villous adenomas were associated with McKittrick–Wheelock syndrome, while in the other groups, this syndrome was

rare. We also found that MUC5AC expression, a mucin normally restricted to the gastric mucosa, was always present in association with this syndrome.

Finally, taken together, clinical and endoscopic data help predict the histological characteristics of at least two groups of villous tumours of the rectosigmoid, i. e. villous adenomas and serrated adenomas.

The pattern of malignant transformation is dictated by the specific histopathological and molecular characteristics of the initial villous tumour

In our series, invasive carcinomas were frequently observed in villous tumours. Interestingly, the invasive tumour maintained the histopathological, molecular and oncogenetic imprint of the parental villous tumour. In fact, the four adenocarcinomas, not otherwise specified, derived from villous or tubulovillous adenomas lacking a serrated component, and the two serrated carcinomas resulted from the malignant transformation of villous tumours with a serrated component. As to whether this is a general rule of transformation remains to be determined in additional cases.

Surprisingly, in our series, all villous tumours and their invasive counterparts shared a MSS phenotype, including those with a serrated component. In addition, most of them displayed a *BRAF* or *KRAS* mutation. This was unexpected, as it is noteworthy that most serrated adenomas or serrated adenocarcinomas are generated through a mutator pathway, characterized by the MSI phenotype.^{18,19} The relatively high frequency of *KRAS* mutations could be in part linked to the loss of *MGMT* expression through promoter methylation, which is known to be associated with G > A mutations in *KRAS*.²⁰ *BRAF* mutation was associated with two of the villous tumours with invasive component and serrated pattern, thus supporting its poor prognostic value in tumours with MSS phenotype. These patterns might have a therapeutic and prognostic impact in the management of villous tumours with malignant behaviour. However, there are several limitations to this retrospective study, due to the relatively small number of tumours, from a single institution. Therefore, this feasibility study needs to be extended in a prospective multi-centre cohort.

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Conflict of interest

The authors declare no conflicts of interest.

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References

1. Quénu E and Landel G. Des tumeurs villeuses ou épithéliomas superficiels végétants du rectum. *Rev de Gynec et de Chi Abdom* 1899; 3: 95–126.
2. McKittrick LS and Wheelock FC. Carcinoma of the colon. 1954. *Dis Colon Rectum* 1997; 40: 1494–1496.
3. Morson BC and Sobin LH. *Histological typing of intestinal tumours*. Geneva: World Health Organization, 1976.
4. Longacre TA and Fenoglio-Preiser CM. Mixed hyperplastic adenomatous polyps/serrated adenomas. A distinct form of colorectal neoplasia. *Am J Surg Pathol* 1990; 14: 524–537.
5. Jass JR. Classification of colorectal cancer based on correlation of clinical, morphological and molecular features. *Histopathology* 2007; 50: 113–130.
6. O'Brien MJ, Yang S, Clebanoff JL, et al. Hyperplastic (serrated) polyps of the colorectum: Relationship of CpG island methylator phenotype and K-ras mutation to location and histologic subtype. *Am J Surg Pathol* 2004; 28: 423–434.
7. Hamilton SR, Bosman FT, Boffetta P, et al. Carcinoma of the colon and rectum. In: *WHO classification of tumours of the digestive system*, 4th edn. Lyon: IARC, pp. 134–146.
8. Snover DC, Ahnen DJ, Burt RW, et al. Serrated polyps of the colon and rectum and serrated polyposis. In: *WHO classification of tumours of the digestive system*, 4th edn. Lyon: IARC, pp. 160–165.
9. Yantiss RK, Oh KY, Chen YT, et al. Filiform serrated adenomas: A clinicopathologic and immunophenotypic study of 18 cases. *Am J Surg Pathol* 2007; 31: 1238–1245.
10. The Paris endoscopic classification of superficial neoplastic lesions: Esophagus, stomach, and colon. *Gastrointest Endosc* 2003; 58: S3–S43.
11. Schlemper RJ, Riddell RH, Kato Y, et al. The Vienna classification of gastrointestinal epithelial neoplasia. *Gut* 2000; 47: 251–255.
12. Li J, Wang L, Mamon H, et al. Replacing PCR with COLD-PCR enriches variant DNA sequences and redefines the sensitivity of genetic testing. *Nat Med* 2008; 14: 579–584.
13. Bossard C, Küry S, Jamet P, et al. Delineation of the infrequent mosaicism of KRAS mutational status in metastatic colorectal adenocarcinomas. *J Clin Pathol* 2012; 65: 466–469.
14. Jarry A, Masson D, Cassagnau E, et al. Real-time allele-specific amplification for sensitive detection of the BRAF mutation V600E. *Mol Cell Probes* 2004; 18: 349–352.
15. Suraweera N, Duval A, Reperant M, et al. Evaluation of tumour microsatellite instability using five quasimonomorphic mononucleotide repeats and pentaplex PCR. *Gastroenterology* 2002; 123: 1804–1811.
16. Ogino S, Kawasaki T, Brahmandam M, et al. Precision and performance characteristics of bisulfite conversion and real-time PCR (MethyLight) for quantitative DNA methylation analysis. *J Mol Diagn* 2006; 8: 209–217.
17. Fu B, Yachida S, Morgan R, et al. Clinicopathologic and genetic characterization of traditional serrated adenomas of the colon. *Am J Clin Pathol* 2012; 138: 356–366.
18. Huang CS, Farraye FA, Yang S, et al. The clinical significance of serrated polyps. *Am J Gastroenterol* 2011; 106: 229–405.
19. Mäkinen MJ. Colorectal serrated adenocarcinoma. *Histopathology* 2007; 50: 131–150.
20. Esteller M, Toyota M, Sanchez-Cespedes M, et al. Inactivation of the DNA repair gene O6-methylguanine-DNA methyltransferase by promoter hypermethylation is associated with G to A mutations in K-ras in colorectal tumorigenesis. *Cancer Res* 2000; 60: 2368–2371.