PERGAMON

European Journal of Cancer 38 (2002) 858–866

European Journal of Cancer

www.ejconline.com

Distinction between familial and sporadic forms of colorectal cancer showing DNA microsatellite instability

J.R. Jass[a,*], M.D. Walsh[a], M. Barker[a], L.A. Simms[b], J. Young[b], B.A. Leggett[b]

[a]*Department of Pathology, University of Queensland, Herston, Queensland 4006, Australia*
[b]*Conjoint Gastroenterology Laboratory, Royal Brisbane Hospital Research Foundation, Queensland, Australia*

Received 18 December 2001; accepted 30 January 2002

Abstract

Attempts to classify colorectal cancer into subtypes based upon molecular characterisation are overshadowed by the classical stepwise model in which the adenoma-carcinoma sequence serves as the morphological counterpart. Clarity is achieved when cancers showing DNA microsatellite instability (MSI) are distinguished as sporadic MSI-low (MSI-L), sporadic MSI-high (MSI-H) and hereditary non-polyposis colorectal cancer (HNPCC). Divergence of the 'methylator' pathway into MSI-L and MSI-H is at least partly determined by the respective silencing of *MGMT* and *hMLH1*. Multiple differences can be demonstrated between sporadic and familial (HNPCC) MSI-H colorectal cancer with respect to early mechanisms, evolution, molecular characterisation, demographics and morphology. By acknowledging the existence of multiple pathways, rapid advances in the fields of basic and translational research will occur and this will lead to improved strategies for the prevention, early detection and treatment of colorectal cancer.

Keywords: Colon; Cancer; Familial; Sporadic; Microsatellite instability; HNPCC

1. Introduction: the classical pathway to colorectal cancer

Mutation of *APC* may occur in the germline and give rise to the autosomal dominant condition familial adenomatous polyposis (FAP) or it may occur somatically and give rise to sporadic neoplasms through the adenoma–carcinoma sequence [1]. Neoplasms complicating FAP have therefore been assumed to represent the familial counterparts of sporadic or common colorectal cancer. The only difference would be that neoplasia in FAP was 'one step ahead' by virtue of the first mutation existing in the germline. A subset of 'sporadic' colorectal cancer may in fact be caused by low penetrant *APC* polymorphisms in the germline, for example, at I1307K and E1317Q [2]. The magnitude of such occult 'familial' disease representing a 'bridge' between classical FAP and sporadic colorectal cancer is unknown.

FAP has therefore served as the model for the well established pathway to colorectal cancer implicating alteration of *APC* followed by K-*ras*, genes on chromosome 18q and *TP53* [3]. Importantly, these genetic alterations are demonstrated at the stage of adenoma and reinforce the adenoma–carcinoma concept. *APC*, *DCC* and *TP53* are regarded as tumour suppressor genes. Inactivation of the second copy is often by non-disjunctional loss detected as loss of heterozygosity (LOH). LOH is correlated with chromosomal aneuploidy, but is also documented in diploid cancers that are DNA microsatellite stable [4,5]. Chromosomal instability is regarded as a prerequisite for the stepwise development of the 'suppressor' pathway in which mutation of *APC* is the initiating event [6]. This has come to be viewed as the dominant evolutionary paradigm for colorectal cancer. The possibility of alternative routes of pathogenesis has received comparatively little consideration [7].

In 1993, it was shown that hereditary non-polyposis colorectal cancer (HNPCC) was caused by germline mutation in a DNA mismatch repair gene. The two most frequently affected genes are *hMSH2* and *hMLH1*. Less frequently implicated are *hMSH6* and *hPMS2* [8–13]. The biomarker for DNA repair deficiency is widespread frameshift mutation evidenced by replication

* Corresponding author. Tel.: +61-7-3365-5340; fax: +61-7-3365-5511.
E-mail address: j.jass@mailbox.uq.edu.au (J.R. Jass).

0959-8049/02/$ - see front matter
PII: S0959-8049(02)00041-2

errors (RERs) in microsatellite markers, and known also as the 'mutator' phenotype or more usually now as DNA microsatellite instability (MSI) [14–16]. Among colorectal cancers showing MSI, only a small subset occurs in the context of HNPCC; most examples are sporadic. It has been suggested that DNA instability does not necessarily initiate a novel pathway, but its early establishment would accelerate or act as a promoting influence within the context of the classical pathway [17–19]. This view fits with the demonstration of MSI at the stage of adenoma in HNPCC [20,21]. Nevertheless, frameshift mutation in a short repetitive sequence would be expected to be a frequent type of somatic mutation in view of the nature of the underlying DNA repair defect. Indeed, it has been shown that certain genes with short mononucleotide encoding runs are targeted in place of the traditional genetic steps [22]. However, a foremost example, *TGFbetaRII*, is also mutated in a proportion of non-MSI+ colorectal cancers [23].

2. A second pathway: fact or fiction

In recent years, some reports have reinforced the concept of a single dominant pathway driven either by chromosomal instability or by DNA instability [24–26]. In this single pathway model, the type of instability might influence the profiles of genetic changes to a mild degree, but not sufficiently to allow the recognition of two independent pathways. Others argue that sporadic MSI+ cancers represent a distinct entity with a unique profile of genetic changes and differing from microsatellite stable (MSS) cancers in terms of older age at presentation, preponderance amongst females, proximal colon predilection, tendency to multiplicity, reduced metastatic potential, increased responsiveness to adjuvant chemotherapy and several light microscopic differences including poor differentiation, mucinous differentiation and lymphocytic infiltration [15,27–37]. What is the basis for these opposing views? Methodological explanations relate to the fact that cancers showing DNA microsatellite instability fall into three groups that must be recognised and studied separately: (1) cancers with high level MSI (MSI-H), (2) cancers with low level MSI (MSI-L) and (3) familial (HNPCC) cancers that are MSI-H. The important distinction of MSI-L versus MSI-H cancer will be briefly reviewed before turning to the main topic of this paper.

3. Microsatellite instability: low versus high

When a series of colorectal cancers is studied with a set of DNA microsatellite markers, those showing DNA microsatellite instability are distributed bimodally with a breakpoint at around 30–40% [28,32,38]. Cancers with instability in less than 30–40% of markers are termed as MSI-L and those with higher levels as MSI-H. Mononucleotide markers are relatively stable and non-polymorphic. Nevertheless, bandshifts occur with high frequency (around 80%) in such markers as BAT25, BAT26, BAT34 and BAT40 in MSI-H cancers. In MSI-L cancers, instability is essentially restricted to dinucleotide and longer repeat markers [38]. Despite this important qualitative difference between the MSI-L and MSI-H status, many studies have included only dinucleotide markers and have diagnosed MSI-H when bandshifts are observed in only two of a panel of eight or more markers. The widely advocated National Cancer Institute (NCI) panel includes three dinucleotide markers and two mononucleotide markers [39]. The dinucleotide markers were selected because of their intrinsic instability and sensitivity to MSI-L status. Using this panel, one is entitled to label a cancer as MSI-H if bandshifts are observed in two dinucleotide markers and no mononucleotide markers. It is questionable if such a cancer is genuinely MSI-H.

MSI-L cancers show a genetic profile similar to MSS, but not MSI-H cancers [40,41]. K-*ras* mutation, *TP53* mutation and LOH at 5q, 17p and 18q are frequent genetic alterations. MSI-L cancers are mainly left sided and are at least as aggressive as MSS cancers [28]. A likely mechanism for MSI-L status is methylation and silencing of the DNA repair gene *O-6-methylguanine DNA methyltransferase* (*MGMT*) on the basis that impaired removal of pro-mutagenic methylguanine adducts is likely to overwhelm an intact DNA mismatch repair system [42]. MSI-L cancers are fundamentally different from MSI-H cancers. Failure to distinguish the two groups adequately provides at least a partial explanation for the insistence by some that MSI+ cancers do not represent a distinct clinicopathological entity, but this is not the whole explanation. As discussed below, failure to distinguish between familial and sporadic forms of MSI-H cancer is likely to be a confounder of similar magnitude.

4. Defining hereditary non-polyposis colorectal cancer

Over the years, an autosomal dominant form of inherited bowel cancer came to be distinguished from FAP by the lack of premonitory signs (adenomas), association with extracolonic neoplasia (uterus, stomach, ovary, small intestine, pelviureter, brain and skin), predilection for proximal colon and a number of histopathological features [43]. The Lynch syndrome was renamed as hereditary non-polyposis colorectal cancer (HNPCC) and the Amsterdam criteria were developed to facilitate the recognition of such families [44,45]. Some groups assumed that all autosomal dominant

kindreds that fulfilled the Amsterdam could be grouped under the term HNPCC. However, when cancers were tested for MSI, only those families associated with MSI-H cancers showed the features described by Lynch [46]. In families fulfilling the Amsterdam criteria, but lacking MSI-H cancers the onset of colorectal cancer is at an older age, and there is less cancer multiplicity, no predilection for the proximal colon, more numerous adenomas and a lack of histological features of MSI-H cancers (poor or mucinous differentiation and lymphocytic infiltration) [46,47]. Naturally enough, germline mutations in DNA mismatch repair genes can only be detected in families with MSI-H cancers [48]. HNPCC is now used in the restricted sense implying an inherited tendency to form cancers that are DNA repair-deficient and not in a vague and confusing sense encompassing all non-FAP colorectal cancer kindreds.

It was originally thought that an inherited basis might underlie the development of all MSI-H colorectal cancers. HNPCC would then account for about 15% of colorectal cancer. It soon became apparent that most MSI-H cancers were sporadic and HNPCC was thought to account for around 5% of bowel cancer. Population-based studies in Finland found a lower figure of 2% [49]. Given that the Finnish population includes very large founder families with HNPCC and is not a high risk country for colorectal cancer, even a 2% figure could be inflated. Population-based studies in high-risk regions for colorectal cancer suggest that the frequency of HNPCC is less than 1% of all bowel cancer [50]. Population-based studies also turn up single case families in which the traditional Lynch features of young age at onset and proximal location may be lacking [50]. In other words, full penetrance and pleiotropism is only observed in classical HNPCC families. 'Sporadic' cases that are in fact due to a germline mutation may therefore be grouped incorrectly with true sporadic cases caused by somatic silencing of a DNA mismatch repair gene. At least 90% of MSI-H colorectal cancers are sporadic.

5. Defining sporadic or non-familial MSI-H colorectal cancer

Absence of a germline mutation in a DNA mismatch repair gene serves as the diagnostic gold standard for sporadic MSI-H colorectal cancer, but for technical reasons proof of an absent mutation is difficult to achieve. Supporting evidence includes absence of a family history of colorectal cancer and presentation at an advanced age. Most sporadic MSI-H cancers are caused by methylation and silencing of the DNA mismatch repair gene *hMLH1* [51,52]. However, methylation of *hMLH1* is not specific, occurring in a subset HNPCC and non-MSI-H colorectal cancers. Immunohistochemical loss of expression of *hMSH2*, *hMSH6* or *hPMS2* in isolation is reasonably strong evidence of a germline mutation in the respective gene, although somatic mutation is not excluded. Since the majority of MSI-H colorectal cancers are sporadic, most studies purporting to distinguish non-familial and HNPCC colorectal cancer are likely to represent a reasonable approximation to the truth, particularly when germline mutation detection has been performed by denaturing gradient gel electrophoresis, denaturing HPLC, direct sequencing and by Southern blot, FISH or haploid analysis to exclude large deletions. Under such circumstances, and when MSI-L cancers have been properly excluded, most investigations into the molecular profile of sporadic MSI-H cancers document the significantly reduced frequency if not absence of mutation in *APC*, K-*ras* and *TP53* and loss of heterozygosity at 5q, 17p and 18q [29,33,36,40,41]. Instead, mutations are described in *TGFbetaRII*, *IGF2R*, *BAX*, *MSH3*, *MSH6*, *caspase* 5, *E2F-4*, *Tcf-4*, *BCL-10*, *cdx-2*, *axin* and *hRAD50* [22,33,53–61]. Additionally, a number of normally functioning genes are silenced by methylation. These include *HPP1* and *p16* as well as *hMLH1* [62,63]. Most sporadic MSI-H cancers show the CpG island methylator phenotype (CIMP) characterised by widespread DNA hypermethylation [62].

Within the category of MSI-H colorectal cancer, HNPCC and sporadic cases have been viewed as familial and non-familial counterparts of the same underlying pathological process (analogous to FAP and sporadic microsatellite stable colorectal cancer). There is now evidence that HNPCC and sporadic MSI-H cancers differ in terms of demographic features, histogenesis, molecular profiles and microscopic morphology and should therefore be studied separately. There are two situations in which HNPCC cancers may enrich a series of MSI-H cancers artificially. The first relates to cancer cell lines. In theory, most should be derived from sporadic MSI-H cancers. In practice, one of the principal reasons why colorectal cell lines have been established over the years is to study cancer genetics. Therefore, cancers from subjects in their eighth decade (the mean age of presentation of sporadic MSI-H cancers) are likely to be underrepresented in cell line libraries. MSI-H cell lines should be assumed to be HNPCC-derived, unless proved otherwise. Certainly this would explain the high frequency of *APC* mutations in these cell lines that is not seen in DNA samples obtained from sporadic MSI-H cancers.

The second situation in which HNPCC cancers could inadvertently enrich a series of MSI-H cancers is more speculative. It is possible, however, that sporadic MSI-H cancers are underrepresented in traditionally low risk regions for colorectal cancer, while germline mutations in DNA mismatch repair genes may be less penetrant allowing HNPCC cancers to present at an older age and

as single cases within families. In Japan, the molecular and pathological profiles of 'sporadic' MSI-H cancers are more consistent with HNPCC than genuinely sporadic MSI-H cancers (see below). Specific examples include the relative lack of mucinous differentiation and the presence of mutations in *APC* and *TP53* [25,64]. It has been suggested that large hyperplastic polyps in the proximal are the precursors of sporadic MSI-H cancers [65,66]. Risk factors for large proximal hyperplastic polyps include female gender and high body mass index [67]. Risk factors for MSI-H cancers include smoking and female gender [68,69]. Smoking and obesity are not features of elderly female Japanese and could therefore account for sporadic MSI-H cancers being underrepresented in this population. It is possible, however, that a separate pathway via flat adenoma accounts for sporadic MSI-H cancer in Japan [25,70]. This fits with the low frequency of K-*ras* mutation and high frequency of mutations in *APC* and *TP53* in apparently sporadic examples of MSI-H cancer in Japan [25].

6. Histogenesis of familial versus non-familial MSI-H colorectal cancer

There is good evidence to support the traditional adenoma-carcinoma sequence in HNPCC:

1. Demonstration of adenomas in young subjects with HNPCC [71].
2. Demonstration of early cancer in adenomas (malignant polyps) [72].
3. Demonstration of residual adenoma adjacent to advanced cancer [73].
4. Demonstration of MSI and loss of expression of a DNA mismatch repair gene congruent with the underlying germline mutation in adenomas [21].
5. Prevention of cancer by adenoma removal [74].

Nevertheless, the adenoma–carcinoma differs from the traditional paradigm insofar as individual adenomas show a high rate of conversion to carcinoma and over a relatively short period of time. The clinical evidence for this assertion is the high frequency of interval cancers presenting between regular colonoscopic screening [75], the low adenoma:carcinoma ratio [47] and the high frequency of adenomas with high grade dysplasia and/or villous change [71]. Early establishment of DNA instability is the likely basis for this rapid progression. The earliest morphological change in HNPCC is the microscopic aberrant crypt focus (ACF) which may be hyperplastic or dysplastic. MSI occurs in both types of ACF [76].

Despite the greater frequency of sporadic MSI-H versus HNPCC colorectal cancer, sporadic adenomas showing MSI-H are very uncommon [66,77]. Most, if not all, sporadic adenomas showing MSI-H develop within unsuspected cases of HNPCC [77]. A possible explanation is the late establishment of MSI-H that would then drive the rapid conversion of adenoma to carcinoma. However, this could not be a traditional adenoma since sporadic MSI-H cancers lack genetic changes that are normally found at the stage of adenoma (implicating *APC*, K-*ras* and *TP53*). It is conceivable that methylation of different genes susceptible to this form of silencing could explain the origin of sporadic MSI-H colorectal cancer. This appears to be the case, but the expected changes occur within serrated polyps and not traditional adenomas [65,66,78]. Serrated polyps encompass epithelial polyps of the colorectum showing crypt serration or infolding. These include hyperplastic aberrant crypt foci, hyperplastic polyps, mixed polyps and serrated adenomas. The adenomatous component of a mixed polyp may be either traditional adenoma or serrated adenoma (or both) [79].

Features supporting the origin of sporadic MSI-H cancers within serrated polyps include:

1. Demonstration of MSI-H and loss of immunohistochemical expression of *hMLH1* within serrated polyps [65,66].
2. Demonstration of mutation in *TGFbetaRII*, *IGF2R*, *BAX*, *MSH3* and *MSH6* in serrated polyps [65].
3. Expression of gastric mucin *MUC5AC* by serrated polyps and sporadic MSI-H colorectal cancer [80].
4. Demonstration of serrated polyps and MSI-H colorectal cancer in contiguity [65,81].
5. Overrepresentation of MSI-H colorectal cancer in subjects with hyperplastic polyposis [65].
6. Demonstration of serrated architecture in sporadic MSI-H colorectal cancer [81].

7. Molecular profiles of familial versus non-familial MSI-H colorectal cancer

Given the differences in the early morphogenesis of familial and sporadic MSI-H colorectal cancer, one would expect to observe matching differences in the molecular profiles of the two pathways. Studies including HNPCC cancers and/or MSI-H cell lines show evidence of disruption of the *wnt* signalling pathway as indicated by *APC* mutation [82,83], *beta-catenin* mutation [84,85] or abnormal cytoplasmic and nuclear staining of beta-catenin (data not shown). Studies that focus on sporadic MSI-H colorectal cancer show little evidence of *APC* mutation [29,36], *beta-catenin* mutation [36] or abnormal immunolocalisation of beta-catenin

[41]. Most studies show a trend towards or significant reduction of K-*ras* mutation in sporadic MSI-H cancers [40,41], whereas K-*ras* mutation occurs at a higher frequency in HNPCC [16,33]. These data fit with the differing routes of morphogenesis of familial versus sporadic MSI-H colorectal cancer, specifically the traditional adenoma–carcinoma sequence in the former and an alternative (serrated) route in the latter.

8. Demographics of familial versus non-familial MSI-H colorectal cancer

Although HNPCC has long been known to show a predilection for the proximal colon, up to 40% of cancers present in the left colon and rectum and rectal cancers are a significant complication following total colectomy and ileorectal anastomosis [86]. By contrast, around 90% of sporadic MSI-H cancers occur in the proximal colon [27]. HNPCC colorectal cancer is more common in males, while females are more likely to develop sporadic MSI-H colorectal cancer [69], despite the association with cigarette smoking.[68]. Females are more likely to show methylation of *hMLH1* [69], a key mechanism in the genesis of sporadic MSI-H colorectal cancer. Whereas HNPCC colorectal cancer occurs in young subjects, sporadic MSI-H colorectal cancer is more age-related than either MSS or MSI-L colorectal cancer. The demographic data indicate the fundamental distinction of familial and sporadic MSI-H colorectal cancer.

9. Morphology of familial versus non-familial MSI-H colorectal cancer

Fifty-one primary colorectal cancers from 36 HNPCC families were compared with 18 sporadic MSI-H colonic cancers (data not shown). A germline mutation was found in 16 of the HNPCC families. In the others, the modified Amsterdam criteria were met and at least two neoplasms per family (colorectal carcinoma or adenoma or endometrial carcinoma) were MSI-H and showed loss of expression of the same DNA mismatch repair gene (*hMLH1* in 26 cases and *hMSH2* in 25 cases). The sporadic cases had no family history of colorectal cancer, were elderly (mean age 74 years), and all showed exclusive loss of expression of *hMLH1* by immunohistochemistry. No germline mutations have been found and methylation of *hMLH1* has been demonstrated in the majority of cases.

Multiple statistically significant morphological differences between these strictly defined groups are observed (Table 1). Overall, HNPCC cancers are more like traditional colorectal cancers apart from the higher frequency of lymphocytic infiltration (Fig. 1). This is a feature of both familial and non-familial groups, but a Crohn's-like reaction (Fig. 2) and peritumoral lymphocytes are significantly more frequent in HNPCC cancers.

Sporadic MSI-H cancers are more likely to show two or more subclones. These are distinguished on the basis of grade of differentiation, for example moderate and

Table 1
Morphology of HNPCC versus sporadic MSI-H colorectal cancer

Feature	HNPCC $n=51$	Sporadic MSI-H $n=18$	P value[a]
Expanding	49 (96%)	18 (100%)	0.55
Poor differentiation	16 (31%)	8 (44%)	0.30
Mucinous	7 (14%)	8 (44%)	<0.01
Serrated architecture	1 (2%)	3 (17%)	0.03
Subclones	13 (25%)	12 (67%)	<0.01
Crohn's-like reaction	36 (71%)	8 (44%)	<0.01
Tumour infiltrating lymphocytes	41 (80%)	11 (61%)	0.1
Peritumoral lymphocytes	19 (37%)	2 (11%)	<0.04
Contiguous serrated adenoma	0 (0%)	4 (22%)	<0.01
Contiguous traditional adenoma	8 (16%)	0 (0%)	0.08

[a] Fisher's Exact or Pearson's Chi square test.

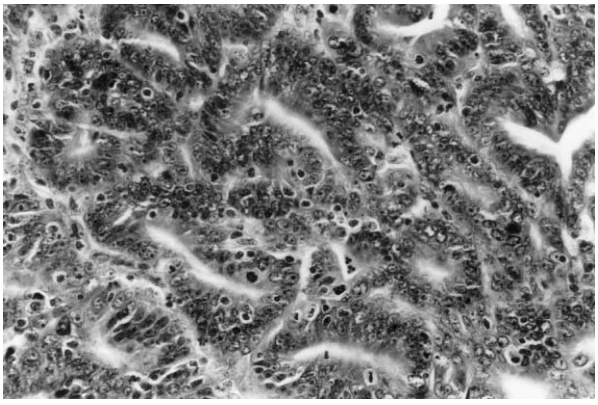

Fig. 1. Tumour infiltrating lymphocytes in a moderately differentiated cancer from a subject with HNPCC. Haematoxylin and eosin.

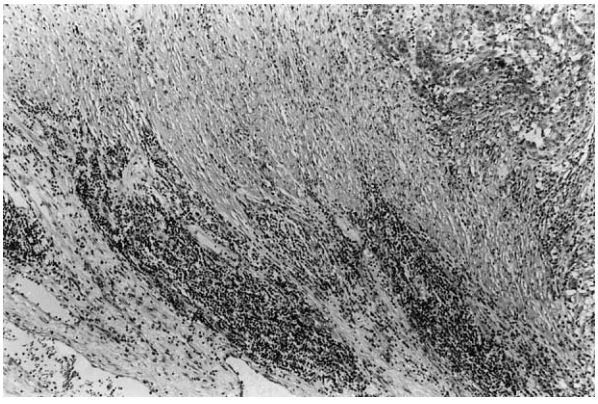

Fig. 2. Crohn's-like reaction (nodules of B-lymphocytes with surrounding T-lymphocytes). Haematoxylin and eosin.

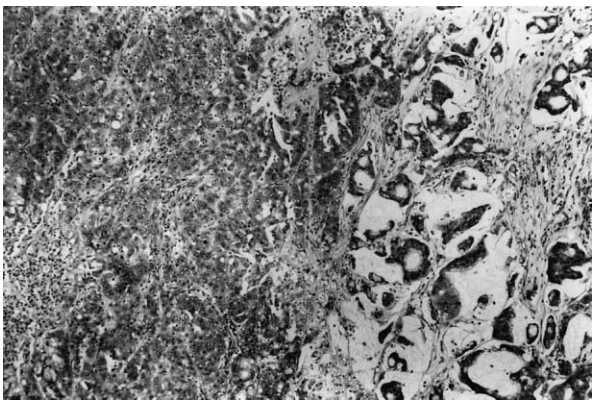

Fig. 3. Poorly differentiated and mucinous subclones side-by-side in a sporadic MSI-H colorectal cancer. Haematoxylin and eosin.

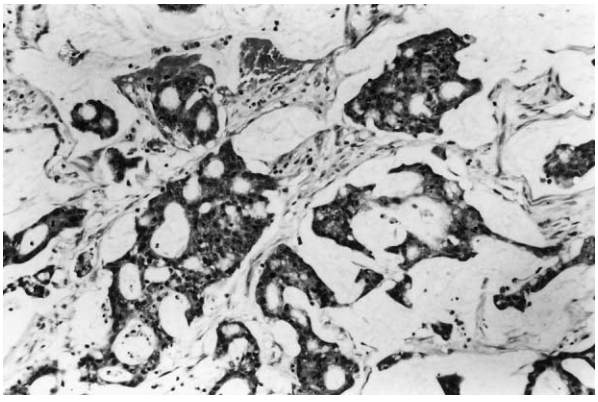

Fig. 4. Moderately to poorly differentiated mucinous adenocarcinoma in which the epithelium is arranged in irregular chains and clusters (same case as Fig. 3). Haematoxylin and eosin.

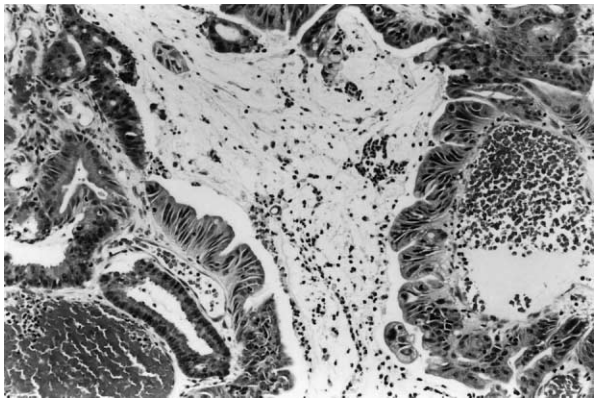

Fig. 5. Residual serrated architecture in a sporadic MSI-H colorectal cancer. Haematoxylin and eosin.

poor (Fig. 3) or type of cancer, for example mucinous and non-mucinous. The mucinous areas in sporadic MSI-H cancers are more likely to be poorly differentiated and composed of ribbons, irregular cell clusters or lace-like structures (Fig. 4). Mucinous change in HNPCC, by contrast, usually features a well differentiated columnar epithelium similar to the epithelium of villous adenoma. Areas showing obvious foci of serration occurred in three sporadic MSI-H cancers (Fig. 5). In these fields and also in non-serrated areas, cytoplasm is typically abundant and eosinophilic, while nuclei are vesicular and contain a prominent nucleolus. Residual serrated adenoma occurred in four sporadic MSI-H cases (Fig. 6), whereas residual traditional adenoma featured in eight HNPCC cancers. The findings reinforce the distinction of familial and sporadic MSI-H colorectal cancer and fit with the histogenetic, molecular and demographic differences discussed above.

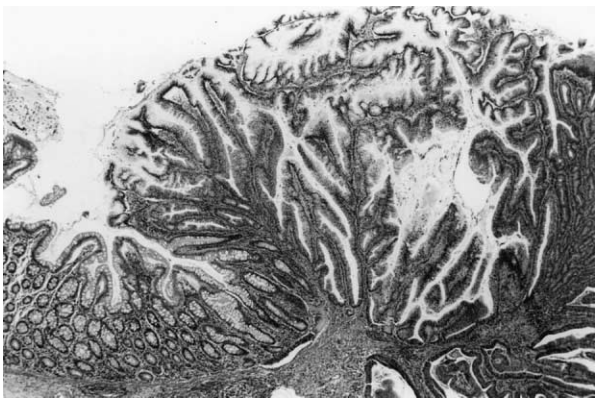

Fig. 6. Serrated adenoma contiguous with a sporadic MSI-H colorectal cancer. Haematoxylin and eosin.

10. Conclusions

The careful separation of MSI-positive colorectal cancer into three subtypes: MSI-H sporadic, MSI-L sporadic and HNPCC, highlights fundamental differences warranting rapid recognition and assimilation into basic and translational research. It is anticipated that these developments will impact upon current approaches to prevention, screening and management of colorectal cancer. The observations raise several questions for future study. Why should sporadic MSI-H cancers evolve through the serrated polyp pathway and not the traditional adenoma–carcinoma sequence as in HNPCC? Given the fact that hyperplastic polyps are especially numerous in the distal colon and rectum, the predilection of sporadic MSI-H colorectal cancer for the proximal colon is paradoxical. Proximal and distal serrated polyps must differ in their modes of pathogenesis. It is expected that the observations will ultimately be explained on the basis of the mutual compatibility of certain combinations of genetic change, but not others (which may be lethal). Additionally, environmental, endogenous or genetic factors will be shown to favour methylation of particular genes and at particular stages of neoplastic progression.

Acknowledgements

This work was supported by the Cooperative Family Registry for Colorectal Cancer Studies, NCI grant 1-U-01-CA74778. I thank B. Mason for secretarial support.

References

1. Solomon E, Voss R, Hall V, *et al*. Chromosome 5 allele loss in human colorectal carcinomas. *Nature* 1987, **828**, 616–619.
2. Frayling IM, Beck NE, Ilyas M, *et al*. The APC variants I1307K and E1317Q are associated with colorectal tumors, but not always with family history. *Proc Natl Acad Sci USA* 1998, **95**, 10722–10727.
3. Vogelstein B, Fearon ER, Hamilton SR, *et al*. Genetic alterations during colorectal-tumor development. *N Engl J Med* 1988, **319**, 525–532.
4. Curtis LJ, Georgiades IB, White S, *et al*. Specific patterns of chromosomal abnormalities are associated with RER status in sporadic colorectal cancer. *J Pathol* 2000, **192**, 440–445.
5. Sugai T, Habano W, Nakamura S, *et al*. Genetic alterations in DNA diploid, aneuploid and multiploid colorectal carcinomas identified by the crypt isolation technique. *Int J Cancer* 2000, **88**, 614–619.
6. Lengauer C, Kinzler KW, Vogelstein B. Genetic instabilities in human cancers. *Nature* 1998, **396**, 643–649.
7. Bond JH. Polyp guideline: diagnosis, treatment, and surveillance for patients with colorectal polyps. *Am J Gastroenterol* 2000, **95**, 3053–3063.
8. Leach FS, Nicolaides NC, Papadopoulos N, *et al*. Mutations of a *mut*S homolog in hereditary nonpolyposis colorectal cancer. *Cell* 1993, **75**, 1215–1225.
9. Fishel R, Lescoe MK, Rao MRS, *et al*. The human mutator gene homolog MSH2 and its association with hereditary nonpolyposis colon cancer. *Cell* 1993, **75**, 1027–1038.
10. Bronner CE, Baker SM, Morrison PT, *et al*. Mutation in the DNA mismatch repair gene homologue *hMLH1* is associated with hereditary non-polyposis colon cancer. *Nature* 1994, **368**, 258–261.
11. Papadopoulos N, Nicolaides NC, Wei Y-F, *et al*. Mutation of a *mutL* Homolog in Hereditary Colon Cancer. *Science* 1994, **263**, 1625–1629.
12. Nicolaides NC, Papadopoulos N, Liu B, *et al*. Mutations of two *PMS* homologues in hereditary nonpolyposis colon cancer. *Nature* 1994, **371**, 75–80.
13. Kolodner RD, Hall NR, Lipford J, *et al*. Structure of the human MLH1 locus and analysis of a large hereditary nonpolyposis colorectal carcinoma kindred for mlh1 mutations. *Cancer Res* 1995, **55**, 242–248.
14. Ionov Y, Peinado MA, Malkhosyan S, Shibata D, Perucho M. Ubiquitous somatic mutations in simple repeated sequences reveal a new mechanism for colonic carcinogenesis. *Nature* 1993, **363**, 558–561.
15. Thibodeau SN, Bren G, Schaid D. Microsatellite instability in cancer of the proximal colon. *Science* 1993, **260**, 816–819.
16. Aaltonen LA, Peltomaki PS, Leach FS, *et al*. Clues to the pathogenesis of familial colorectal cancer. *Science* 1993, **260**, 812–816.
17. Kinzler KW, Vogelstein B. Lessons from hereditary colorectal cancer. *Cell* 1996, **87**, 159–170.
18. Homfray TF, Cottrell SE, Ilyas M, *et al*. Defects in mismatch repair occur after *APC* mutations in the pathogenesis of sporadic colorectal tumours. *Hum Mutat* 1998, **11**, 114–120.
19. Tomlinson IP, Novelli MR, Bodmer WF. The mutation rate and cancer. *Proc Natl Acad Sci USA* 1996, **93**, 14800–14803.
20. Aaltonen LA, Peltomaki P, Mecklin J-P, *et al*. Replication errors in benign and malignant tumours from hereditary nonpolyposis colorectal cancer patients. *Cancer Res* 1994, **54**, 1645–1648.
21. Iino H, Simms LA, Young J, *et al*. DNA microsatellite instability and mismatch repair protein loss in adenomas presenting in hereditary non-polyposis colorectal cancer. *Gut* 2000, **47**, 37–42.
22. Markowitz S, Wang J, Myeroff L, *et al*. Inactivation of the type II TGF-β receptor in colon cancer cells with microsatellite instability. *Science* 1995, **268**, 1336–1338.
23. Grady WM, Myeroff LL, Swinler SE, *et al*. Mutational inactivation of transforming growth factor beta receptor type II in microsatellite stable colon cancers. *Cancer Res* 1999, **59**, 320–324.
24. Tomlinson I, Ilyas M, Johnson V, *et al*. A comparison of the genetic pathways involved in the pathogenesis of three types of colorectal cancer. *J Pathol* 1998, **184**, 148–152.
25. Shitoh K, Konishi F, Miyaki M, *et al*. Pathogenesis of non-familial colorectal carcinomas with high microsatellite instability. *J Clin Pathol* 2000, **53**, 841–845.
26. Curran B, Lenehan K, Mulcahy H, *et al*. Replication error phenotype, clinicopathological variables, and patient outcome in Dukes' B stage II (T3, N0,M0) colorectal cancer. *Gut* 2000, **46**, 200–204.
27. Kim H, Jen J, Vogelstein B, Hamilton SR. Clinical and pathological characteristics of sporadic colorectal carcinomas with DNA replication errors in microsatellite sequences. *Am J Pathol* 1994, **145**, 148–156.
28. Jass JR, Do K-A, Simms LA, *et al*. Morphology of sporadic colorectal cancer with DNA replication errors. *Gut* 1998, **42**, 673–679.
29. Olschwang S, Hamelin R, Laurent-Puig P, *et al*. Alternative genetic pathways in colorectal carcinogenesis. *Proc Natl Acad Sci USA* 1997, **94**, 12122–12127.
30. Messerini L, Vitelli F, de Vitis LR, *et al*. Microsatellite instability in sporadic mucinous colorectal carcinomas: relationship to clinico-pathological variables. *J Pathol* 1997, **182**, 380–384.
31. Iacopetta BJ, Welch J, Soong R, *et al*. Mutation of the transforming growth factor-β type II receptor gene in right-sided colorectal cancer: relationship to clinicopathological features and genetic alterations. *J Pathol* 1998, **184**, 390–395.
32. Thibodeau SN, French AJ, Cunningham JM, *et al*. Microsatellite instability in colorectal cancer: different mutator phenotypes and the principle involvement of *hMLH1*. *Cancer Res* 1998, **58**, 1713–1718.
33. Fujiwara T, Stoker JM, Watanabe T, *et al*. Accumulated clonal genetic alterations in familial and sporadic colorectal carcinomas with widespread instability in microsatellite sequences. *Am J Pathol* 1998, **153**, 1063–1078.
34. Halling KC, French AJ, McDonell SK, *et al*. Microsatellite instability and 8p allelic imbalance in stage B2 and C colorectal cancers. *J Natl Cancer Inst* 1999, **91**, 1295–1303.
35. Cawkwell L, Li D, Lewis FA, *et al*. Microsatellite instability in colorectal cancer: improved assessment using fluorescent polymerase chain reaction. *Gastroenterology* 1995, **109**, 465–471.
36. Salahshor S, Kressner U, Påhlman L, *et al*. Colorectal cancer with and without microsatellite instability involves different genes. *Genes Chromosomes Cancer* 1999, **26**, 247–252.
37. Elsaleh H, Joseph D, Grieu F, Zeps N, Iacopetta B. Association of tumour site and six with survival benefit from adjuvant chemotherapy in colorectal cancer. *Lancet* 2000, **355**, 1745–1750.
38. Dietmaier W, Wallinger S, Bocker T, *et al*. Diagnostic microsatellite instability: definition and correlation with mismatch repair protein expression. *Cancer Res* 1997, **57**, 4749–4756.
39. Boland CR, Thibodeau SN, Hamilton SR, *et al*. A National Cancer Institute Workshop on microsatellite instability for cancer detection and familial predisposition: development of international criteria for the determination of microsatellite instability in colorectal cancer. *Cancer Res* 1998, **58**, 5248–5257.

40. Konishi M, Kikuchi-Yanoshita R, Tanaka K, *et al*. Molecular nature of colon tumors in hereditary nonpolyposis colon cancer, familial polyposis, and sporadic colon cancer. *Gastroenterology* 1996, **111**, 307–317.
41. Jass JR, Biden KG, Cummings M, *et al*. Characterisation of a subtype of colorectal cancer combining features of the suppressor and mild mutator pathways. *J Clin Pathol* 1999, **52**, 455–460.
42. Whitehall V, Walsh MD, Young J, Leggett BA, Jass JR. Methylation of 0-6-methylguanine DNA methyltransferase characterises a subset of colorectal cancer with low level DNA microsatellite instability. *Cancer Res* 2001, **61**, 827–830.
43. Lynch HT, Smyrk T, Lynch JF. Overview of natural history, pathology, molecular genetics and mangement of HNPCC (Lynch syndrome). *Int J Cancer* 1996, **69**, 38–43.
44. Vasen HFA, Mecklin J-P, Khan PM, Lynch HT. The international collaborative group on hereditary non-polyposis colorectal cancer (ICG-HNPCC). *Dis Colon Rectum* 1991, **34**, 424–425.
45. Vasen HF, Watson P, Mecklin J-P, Lynch HT. New clinical criteria for hereditary nonpolyposis colorectal cancer (HNPCC, Lynch syndrome) proposed by the International Collaborative Group on HNPCC. *Gastroenterology* 1999, **116**, 1453–1456.
46. Jass JR, Cottier DS, Jeevaratnam P, *et al*. Diagnostic use of microsatellite instability in hereditary non-polyposis colorectal cancer. *Lancet* 1995, **346**, 1200–1201.
47. Jass JR, Pokos V, Arnold JL, *et al*. Colorectal neoplasms detected colonoscopically in at-risk members of colorectal cancer families stratified by the demonstration of DNA microsatellite instability. *J Mol Med* 1996, **74**, 547–551.
48. Aaltonen LA, Salovaara R, Kristo P, *et al*. Incidence of hereditary nonpolyposis colorectal cancer and the feasibility of molecular screening for the disease. *N Engl J Med* 1998, **338**, 1481–1487.
49. Mecklin J-P, Jarvinen HJ, Hakkiluoto A, *et al*. Frequency of hereditary nonpolyposis colorectal cancer. A prospective multicenter study in Finland. *Dis Colon Rectum* 1995, **38**, 588–593.
50. Peel DJ, Ziogas A, Fox EA, *et al*. Characterization of hereditary nonpolyposis colorectal cancer families from a population-based series of cases. *J Natl Cancer Inst* 2000, **92**, 1517–1522.
51. Herman JG, Umar A, Polyak K, *et al*. Incidence and functional consequences of hMLH1 promoter hypermethylation in colorectal carcinoma. *Proc Natl Acad Sci USA* 1998, **95**, 6870–6875.
52. Kuismanen SA, Holmberg MT, Salovaara R, De La Chapelle A, Peltomäki P. Genetic and epigenetic modification of *MLH1* accounts for a major share of microsatellite-unstable colorectal cancers. *Am J Pathol* 2000, **126**, 1773–1779.
53. Souza RF, Appel R, Yin J, *et al*. The insulin-like growth factor II receptor gene is a target of microsatellite instability in human gastrointestinal tumours. *Nature Genet* 1996, **14**, 255–257.
54. Rampino N, Yamamoto H, Ionov Y, *et al*. Somatic frameshift mutations in the *BAX* gene in colon cancers of the microsatellite mutator phenotype. *Science* 1997, **275**, 967–969.
55. Yamamoto H, Sawai H, Perucho M. Frameshift somatic mutations in gastrointestinal cancer of the microsatellite mutator phenotype. *Cancer Res* 1997, **57**, 4420–4426.
56. Schwartz Jr S, Yamamoto H, Navarro M, *et al*. Frameshift mutations at mononucleotide repeats in *caspase*-5 and other target genes in endometrial and gastrointestinal cancer of the microsatellite mutator phenotype. *Cancer Res* 1999, **59**, 2995–3002.
57. Planck M, Wenngren E, Borg A, Olsson H, Nilbert M. Somatic frameshift alterations in mononucleotide repeat-containing genes in different tumor types from an HNPCC family with germline *MSH2* mutation. *Genes Chromosomes Cancer* 2000, **29**, 33–39.
58. Simms LA, Young J, Wicking C, *et al*. The apoptotic regulatory gene, BCL10, is mutated in sporadic mismatch repair deficient colorectal cancers. *Cell Death Differ* 2000, **7**, 236–237.
59. Wicking C, Simms LA, Evans T, *et al*. *CDX2*, a human homologue of *Drosophila caudal*, is mutated in both alleles in a replication error positive colorectal cancer. *Oncogene* 1998, **17**, 657–659.
60. Liu W, Dong X, Mai M, *et al*. Mutations in *AXIN2* cause colorectal cancer with defective mismatch repair by activating β-catenin/TCF signalling. *Nature Genet* 2000, **26**, 146–147.
61. Kim N-G, Choi YR, Baek MJ, *et al*. Frameshift mutations at coding mononucleotide repeats of the *hRAD50* gene in gastrointestinal carcinomas with microsatellite instability. *Cancer Res* 2001, **61**, 36–38.
62. Toyota M, Ahuja N, Ohe-Toyota M, *et al*. CpG island methylator phenotype in colorectal cancer. *Proc Natl Acad Sci USA* 1999, **96**, 8681–8686.
63. Young JP, Biden KG, Simms LA, *et al*. HPP1: a transmembrane protein commonly methylated in colorectal polyps and cancers. *Proc Natl Acad Sci USA* 2001, **98**, 265–270.
64. Senba S, Konishi F, Okamoto T, *et al*. Clinicopathologic and genetic features of nonfamilial colorectal carcinomas with DNA replication errors. *Cancer* 1998, **82**, 279–285.
65. Jass JR, Iino H, Ruszkiewicz A, *et al*. Neoplastic progression occurs through mutator pathways in hyperplastic polyposis of the colorectum. *Gut* 2000, **47**, 43–49.
66. Jass JR, Young J, Leggett BA. Hyperplastic polyps and DNA microsatellite unstable cancers of the colorectum. *Histopathology* 2000, **37**, 295–301.
67. Jass JR, Young PJ, Robinson EM. Predictors of presence, multiplicity, size and dysplasia of colorectal adenomas. A necropsy study in New Zealand. *Gut* 1992, **33**, 1508–1514.
68. Slattery ML, Curtin K, Anderson K, *et al*. Associations between cigarette smoking, lifestyle factors, and microsatellite instability in colon tumors. *J Natl Cancer Inst* 2000, **92**, 1831–1836.
69. Malkhosyan SR, Yamamoto H, Piao Z, Perucho M. Late onset and high incidence of colon cancer of the mutator phenotype with hypermethylated *hMLH1* gene in women. *Gastroenterology* 2000, **119**, 598.
70. Sasaki S, Masaki T, Umetani N, *et al*. Microsatellite instability is associated with the macroscopic configuration of neoplasms in patients with multiple colorectal adenomas. *Jpn J Clin Oncol* 1998, **28**, 427–430.
71. Jass JR, Stewart SM, Stewart J, Lane MR. Hereditary non-polyposis colorectal cancer: morphologies, genes and mutations. *Mutat Res* 1994, **290**, 125–133.
72. Love RR. Adenomas are precursor lesions for malignant growth in non-polyposis hereditary carcinoma of the colon and rectum. *Surg Gynecol Obstet* 1986, **162**, 8–12.
73. Jass JR. Colorectal adenomas in surgical specimens from subjects with hereditary non-polyposis colorectal cancer. *Histopathology* 1995, **27**, 263–267.
74. Järvinen HJ, Aarnio M, Mustonen H, *et al*. Controlled 15-year trial on screening for colorectal cancer in families with hereditary nonpolyposis colorectal cancer. *Gastroenterology* 2000, **118**, 829–834.
75. Vasen HFA, Nagengast FM, Meera Khan P. Interval cancers in hereditary non-polyposis colorectal cancer (Lynch syndrome). *Lancet* 1995, **345**, 1183–1184.
76. Pedroni M, Sala E, Scarselli A, *et al*. Microsatellite instability and mismatch-repair protein expression in hereditary and sporadic colorectal carcinogenesis. *Cancer Res* 2001, **61**, 896–899.
77. Loukola A, Salovaara R, Kristo P, *et al*. Microsatellite instability in adenomas as a marker for hereditary nonpolyposis colorectal cancer. *Am J Pathol* 1999, **155**, 1849–1853.
78. Iino H, Jass JR, Simms LA, *et al*. DNA microsatellite instability in hyperplastic polyps, serrated adenomas, and mixed polyps: a mild mutator pathway for colorectal cancer? *J Clin Pathol* 1999, **52**, 5–9.

79. Jass JR. Serrated adenoma and colorectal cancer. *J Pathol* 1999, **187**, 499–502.
80. Biemer-Hüttmann A-E, Walsh MD, McGuckin MA, *et al*. Mucin core protein expression in colorectal cancers with high levels of microsatellite instability indicates a novel pathway of morphogenesis. *Clin Cancer Res* 2000, **6**, 1909–1916.
81. Mäkinen MJ, George SMC, Jernvall P, *et al*. Colorectal carcinoma associated with serrated adenoma—prevalence, histological features, and prognosis. *J Pathol* 2001, **193**, 286–294.
82. Huang J, Papadopoulos N, McKinley AJ, *et al*. APC mutations in colorectal tumors with mismatch repair deficiency. *Proc Natl Acad Sci USA* 1996, **93**, 9049–9054.
83. Rowan AJ, Lamlum H, Ilyas M, *et al*. *APC* mutations in sporadic colorectal tumors: a mutational ''hotspot'' and interdependence of the ''two hits''. *Proc Natl Acad Sci USA* 2000, **97**, 3352–3357.
84. Mirabelli-Primdahl L, Gryfe R, Kim H, *et al*. Beta-catenin mutations are specific for colorectal carcinomas with microsatellite instability but occur in endometrial carcinomas irrespective of mutator pathway. *Cancer Res* 1999, **59**, 3346–3351.
85. Miyaki M, Iijima T, Kimura J, *et al*. Frequent mutation of *β-catenin* and *APC* genes in primary colorectal tumors from patients with hereditary nonpolyposis colorectal cancer. *Cancer Res* 1999, **59**, 4506–4509.
86. Rodriguez-Bigas MA, Vasen HFA, Pekka-Mecklin J, *et al*. Rectal cancer risk in hereditary nonpolyposis colorectal cancer after abdominal colectomy. *Ann Surg* 1997, **225**, 202–207.