American Journal of Pathology, Vol. 157, No. 2, August 2000

Colorectal Carcinomas Arising in the Hyperplastic Polyposis Syndrome Progress through the Chromosomal Instability Pathway

Nicholas J. Hawkins,* Patricia Gorman,† Ian P. M. Tomlinson,‡ Peter Bullpitt,§ and Robyn L. Ward¶

From the School of Pathology, University of New South Wales, Sydney, Australia; the Human Cytogenetics Laboratory,† Imperial Cancer Research Fund, London, United Kingdom; the Molecular and Population Genetics Laboratory,‡ Imperial Cancer Research Fund, London, United Kingdom; the Department of Anatomical Pathology,§ Prince of Wales Hospital, Randwick, Australia; and St. Vincent's School of Medicine,¶ University of New South Wales, Sydney, Australia*

The hyperplastic polyposis syndrome is characterized by the presence within the colon of multiple large hyperplastic polyps. We describe a case of hyperplastic polyposis syndrome associated with two synchronous carcinomas, one of which arises within a pre-existing hyperplastic lesion. Comparative genomic hybridization was used to determine genetic changes in both carcinomas and several associated hyperplastic lesions. Microsatellite analysis at five loci was performed on carcinomas and representative hyperplastic polyps, and p53 status was analyzed by immunohistochemistry. Both carcinomas showed multiple genetic aberrations, including high level gains of 8q and 13q, and loss of 5q. These changes were not seen in the hyperplastic polyps. Microsatellite instability was not seen in the carcinomas, four separate hyperplastic polyps, the hyperplastic polyp with mild adenomatous change associated with the carcinoma, or a separate serrated adenoma. Allelic imbalance in the cancers at D5S346 and D17S938 suggested allelic loss of both *p53* and *APC*, as well as at the loci D13S263, D13S174, D13S159, and D18S49. An early invasive carcinoma in one hyperplastic polyp stained for p53 protein, but the associated hyperplastic polyp was negative. In this case, neoplastic progression followed the typical genetic pathway of common colorectal carcinoma and occurred synchronously with mutation of *p53*. *(Am J Pathol 2000, 157:385–392)*

Over the last decade, our understanding of the genetic basis of colorectal tumor development has greatly increased. There has been increasing recognition of a distinct pathway of colorectal carcinogenesis, characterized by sporadic or hereditary defects in DNA repair enzymes and defined genetically by microsatellite instability. This pathway has been variously termed the RER+, microsatellite instability, or mutator phenotype pathway. Importantly, it has thrown into relief the more typical pathway of colorectal cancer progression (referred to hereafter as common colorectal carcinoma), that is characterized, *inter alia*, by early loss of adenomatous polyposis coli (APC), mutations of *p53*, and chromosomal instability.[1] An important first step in the description of these pathways was the recognition of unusual clinicopathological variants of colorectal cancer, such as hereditary nonpolyposis colorectal cancer and familial adenomatous polyposis. For it was analysis of these variants that allowed identification of the key genetic events that underpinned them.

The hyperplastic polyposis syndrome (HPS) is an uncommon condition, characterized by the presence within the colon and rectum of multiple hyperplastic polyps. It was first described as a discrete entity in 1980,[2] and some 49 cases have been reported in the literature to date. This includes those cases reviewed by Jorgensen et al in 1996,[3] together with more recent reports by others.[4–9] Torlakovic and Snover have also described a potential variant of HPS, which they have termed the serrated adenomatous polyposis syndrome.[10]

This combined literature emphasizes the phenotypic differences between the polyps seen in HPS and the common sporadic hyperplastic polyp. Clearly, an important feature is multiplicity, and various reports have described from 12 to more than 100 polyps in HPS. A second feature is the increased size of individual polyps; lesions with a maximum dimension of 10 mm are quite common. In contrast, sporadic polyps are only occasionally greater than 5 mm in size.[2] Another important characteristic of the hereditary syndrome is the distribution of hyperplastic lesions in the colon. Sporadic hyper-

Supported in part by the St Vincent's Clinic Foundation Travelling Fellowship and by the Macquarie Bank–Royal Australian College of Physicians Fellowship (R.L.W.).

Accepted for publication April 27, 2000.

Address reprint requests to Prof. Robyn L. Ward, Department of Medical Oncology, St. Vincent's Hospital, Victoria St., Darlinghurst, NSW 2010, Australia. E-mail: r.ward@garvan.unsw.edu.au.

plastic polyps are seen mainly in the rectum and sigmoid colon, whereas lesions in HPS are distributed in a relatively even fashion throughout the length of the large bowel. Finally, a particular feature of the polypoid lesions in the HPS is their tendency to show atypical cytological features, while maintaining the serrated architecture of the hyperplastic polyp. In the context of sporadic lesions, this combination is variously referred to as a mixed hyperplastic polyp or serrated adenoma.[11] Although seen only rarely in sporadic lesions, it has been reported to a greater though variable extent in HPS,[8,10] often as foci of adenomatous dysplasia.[4,12–14]

It has been suggested that the atypia seen in sporadic serrated adenomas reflects neoplastic progression in an otherwise hyperplastic or metaplastic lesion and that such lesions may represent an alternative pathway to colorectal carcinogenesis.[15] Not surprisingly, the finding of multiple serrated adenomas in the setting of HPS has led to speculation that individuals with this condition also share a significant increase in cancer risk that is analogous to the increased risk seen in adenomatous polyposis.[4,8] This contention is supported by the fact that 18 of the 49 cases (35%) of hyperplastic polyposis or serrated adenomatous polyposis reported in the literature have been associated with synchronous colorectal carcinomas.

However, ascertainment bias will certainly favor the identification of HPS in the presence of synchronous carcinoma. The possibility remains that carcinoma in the HPS may not arise from serrated adenomas or hyperplastic polyps, but rather from unrecognized adenomas of the usual type, or indeed *de novo*. One carcinoma reported in an individual with HPS was shown to have arisen in an otherwise typical tubular adenoma.[16] Furthermore, although cases of adenocarcinoma have been reported as actually arising within sporadic hyperplastic polyps and serrated adenomas,[17,18] to date this phenomenon has not been reported in individuals with the HPS syndrome.

If carcinomas do arise within a morphologically distinctive precursor lesion, such as those of HPS, then this raises the intriguing possibility that the lesions may reflect hitherto unrecognized genetic alterations within colonic epithelial cells. Furthermore, these alterations may be important in the development of other, more common forms of colorectal carcinoma. Jass has pursued this issue and has highlighted a number of the biochemical and genetic differences that he considers to be typical of what he has termed the "mild mutator" pathway,[19] including low-level microsatellite instability. Yet to date, information on genetic changes in HPS and the carcinomas that arise within it remain limited, largely because of the relative rarity of the syndrome.

In this report, we describe a case of the hyperplastic polyposis syndrome associated with two synchronous carcinomas of different stages, one of which shows clear histological evidence of origin within a pre-existing hyperplastic lesion. We also present a cytogenetic and molecular analysis of the carcinomas and representative premalignant lesions from this individual, in an effort to shed light on the genetic changes that lead to the development of carcinoma in this syndrome.

Case Report, Materials and Methods

Report of Case

A 73-year-old man presented with acute bowel obstruction of 2 days' duration. He had previously been well and had no past or family history of colorectal carcinoma or other bowel diseases. Investigation revealed a constricting lesion of the descending colon, and he underwent an emergency subtotal colectomy. His postoperative course was uneventful.

The obstructing lesion was a large, infiltrative and poorly differentiated adenocarcinoma (45 × 80 mm) that encircled most of the bowel wall and extended 15 mm through the bowel wall, with extensive infiltration of the serosa (T4). Of the 13 pericolic and regional lymph nodes identified within the descending colon, nine were involved by tumor. No hepatic or other distant metastases were reported at the time of operation, and the tumor was staged as Dukes stage C (TNM stage III: T4, N1, M0, RO).

A second, smaller adenocarcinoma was identified within the transverse colon, 200 mm proximal to the first tumor. It was an ulcerating lesion (10 × 8 mm) and invaded the bowel wall to a depth of 3 mm. It had an exophytic margin of pale tissue, up to 3 mm in thickness. Histological examination (Figure 1, A and B) revealed a moderately differentiated adenocarcinoma invading the submucosa, without evidence of adjacent involved lymph nodes (Dukes stage A; TNM stage I: T1, N0, M0, R0). The rim of pale tissue surrounding the tumor had the appearance of hyperplastic epithelium and focally showed features suggestive of adenomatous transformation (Figure 1, B and C).

Approximately 75 separate mucosal polyps were distributed randomly but evenly throughout the descending, transverse, and ascending colon, as well as the cecum. They varied in size from 2 to 10 mm, and eight lesions were greater than 8 mm in diameter. One lesion was seen to arise separately from, but in continuity with, the smaller tumor, and several lesions were within 10 mm of the margins of the larger tumor. The larger lesions were pale, with a soft texture and coarsely wrinkled surface, and did not appear macroscopically to be typical of tubular adenomas. On microscopic examination, nearly all of the larger lesions and all representative smaller ones showed a typical hyperplastic architecture (Figure 2A). However, one of the discrete polyps examined (4 × 3 mm) showed the typical features of a serrated adenoma (Figure 2B). A typical hyperplastic polyp was also noted within the appendix.

Tissues and DNA

Before processing, representative tissue samples were removed from the larger carcinoma (T1) and four representative hyperplastic polyps (HP1–4), as well as from normal mucosa (N) and an involved lymph node adjacent to the large tumor (LN). DNA was extracted from these tissues by standard methods. In the case of the smaller carcinoma (T2), its associated hyperplastic polyp (HP-T), and the separate serrated lesion (SA), relevant neoplastic

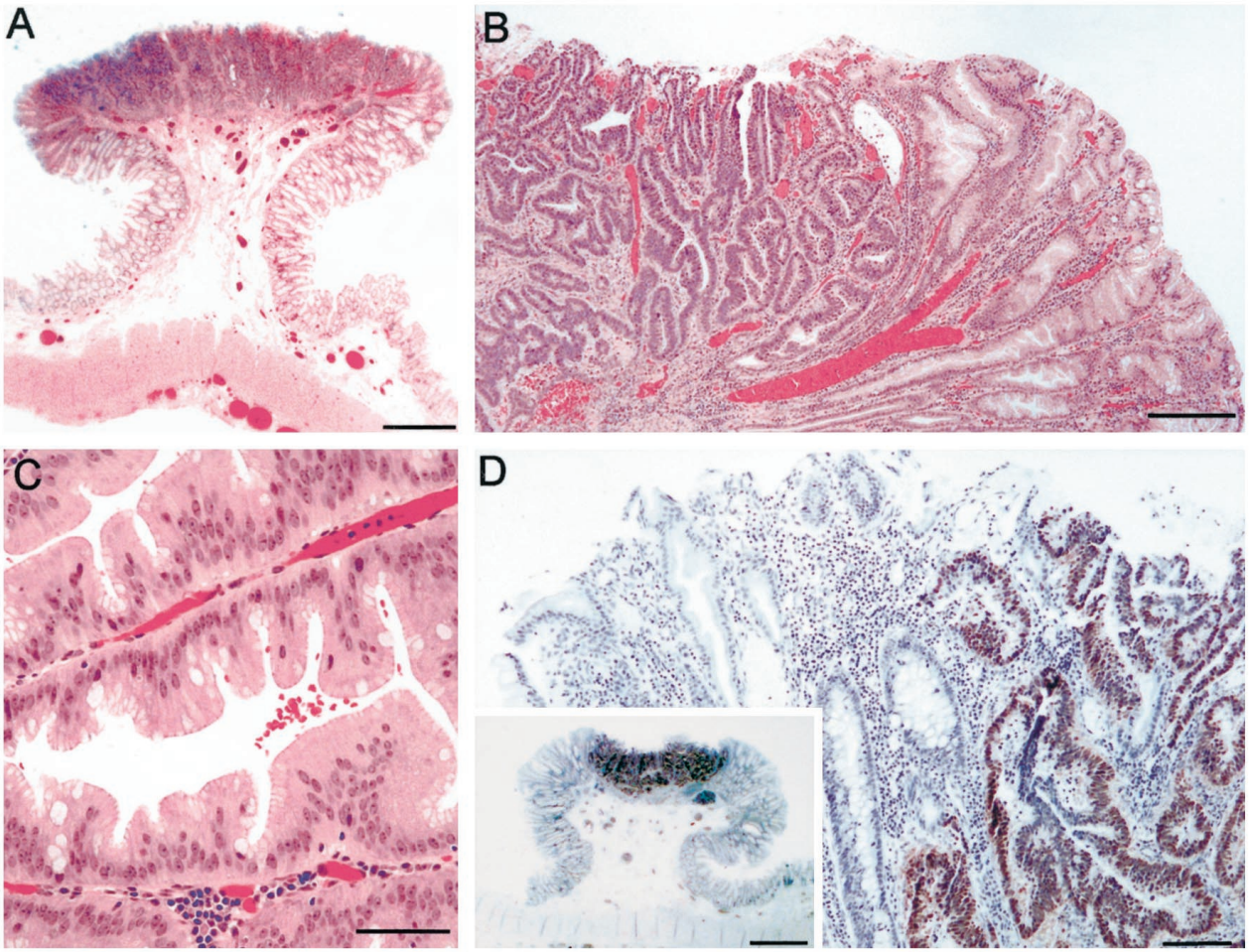

Figure 1. Histological and immunohistochemical appearance of the small carcinoma and its associated hyperplastic lesion. **A:** A scanning power view shows the polypoid nature of the smaller carcinoma (T2), with a central infiltrative lesion and surrounding hyperplastic epithelium. **B:** The same lesion is shown at higher magnification, in which the close proximity of carcinoma and hyperplastic-type epithelium can be seen. **C:** The mildly adenomatous morphology of much of the lesion (HP-T) surrounding the small carcinoma highlights the dense eosinophilic cytoplasm and mild nuclear and architectural atypia seen focally in this lesion. Hematoxylin and eosin. Bars represent 2 mm (**A**), 400 μm (**B**), and 50 μm (**C**). **D:** p53 immunostaining shows strong positivity for the carcinoma (brown staining), in contrast to the surrounding hyperplastic lesion and normal mucosa. A high-power view of the interface between carcinoma and hyperplastic lesion is shown (bar = 150 μm), and a scanning-power view of the entire lesion is shown in the **inset** (bar = 2 mm). Hematoxylin counterstain.

tissues were microdissected from 12 toludine blue-stained tissue sections (10 μm) cut from paraffin-embedded blocks. After extraction of paraffin by xylene and ethanol, DNA was extracted with a Qiamp Tissue kit (Qiagen, Germany), according to the instructions of the manufacturer.

Comparative Genomic Hybridization

Comparative genomic hybridization (CGH) was performed using directly labeled fluorochrome-conjugated DNA. Samples were analyzed on at least three occasions and were also cohybridized against normal mucosal DNA (N), as well as unrelated normal male DNA. Briefly, tissue samples (T1, T2, LN, HP1) and normal DNA were labeled by nick translation,[20] but DNA from other lesions was of insufficient quality for CGH analysis. Fluoroscein isothiocyanate-labeled DNA from each lesion and Texas red-labeled reference DNA (500 ng) were precipitated in the presence of 50 μg Cot-I DNA (Life Technologies, Gaithersburg, MD), sodium acetate, and ethanol. The probes were dissolved in 10 μl of hybridization buffer (50% formamide, 10% dextran sulfate, 1% Tween 20, 2× standard saline citrate, pH 7.0), denatured by heating to 75°C for 5 minutes, and then allowed to preanneal at 37°C for 30 minutes. The mixture was then applied to denatured metaphase slides of male human lymphocytes (Vysis, Downers Grove, IL), and the slides were incubated at 37°C for 72 hours. After washing in 50% formamide/2× standard saline citrate, ethanol dehydration, and air drying, they were counterstained with 4,6-diamidino-2-phenylindole. Images were captured with a cooled charge-coupled device camera attached to a Zeiss Axioskop microscope. Images from five to nine metaphases were analyzed with the Quantitative Image Processing System (QUIPS; Vysis). A chromosome region was considered to be gained if the mean hybridization ratio between the test and normal samples was >1.2:1, and ratios of <0.8:1

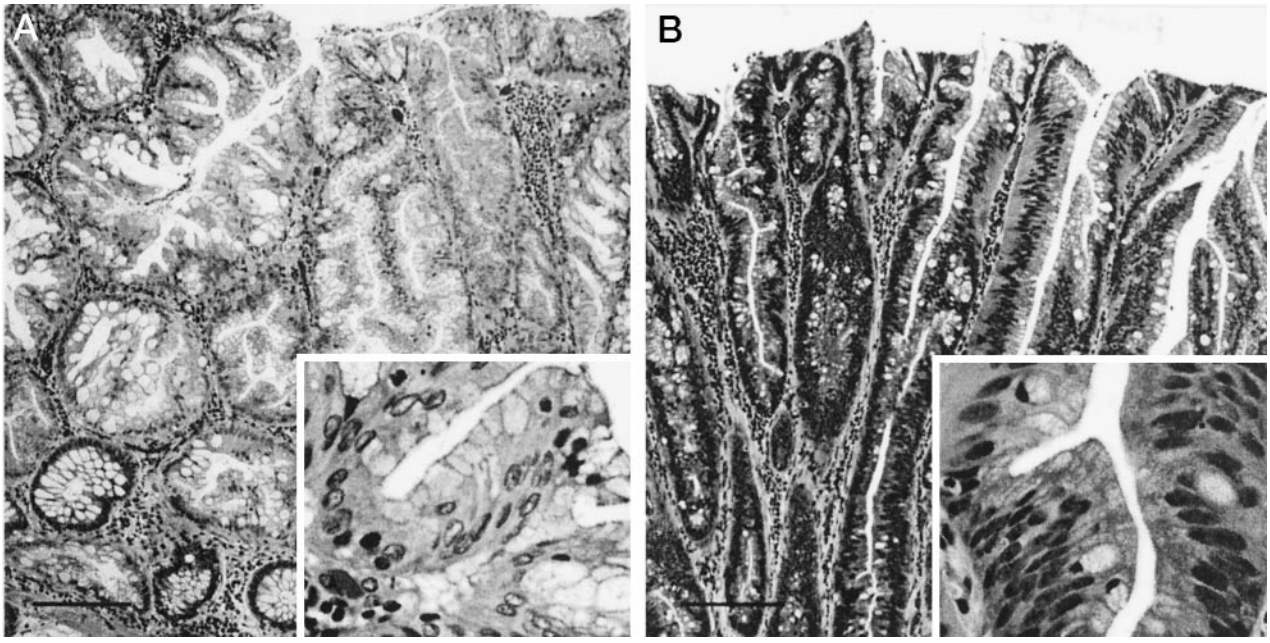

Figure 2. Microscopic features of a typical hyperplastic polyp (**A**) and the single serrated adenoma (**B**) seen in the resected colon. For each micrograph, the bar represents 200 μm, and the **inset** (**bottom right** of each micrograph) shows a fourfold magnification of the same lesion. Hematoxylin and eosin.

represented loss. High-level gain was defined as a ratio of >1.5:1 or as discrete spots of green signal. Negative control hybridizations were included in all experiments.

Polymerase Chain Reaction Analysis for Microsatellite Instability and Allelic Imbalance

Polymerase chain reaction amplification of microsatellite loci, using primers for D5S346, D2S123, Bat 26, Bat 25, and D17S938, was used to determine the microsatellite status[21] of all lesions (T1, T2, LN, HP-T, SA, HP1–4) as compared with normal mucosal DNA from the same individual. In addition, DNA from a subset of samples (T1, T2, HP1) was amplified using the primers D13S263, D13S174, D13S159, and D18S49 to further evaluate some of the abnormalities detected by CGH. In all reactions, DNA was amplified in a 10-μl reaction containing 3 μl of DNA, 200 μmol/L dNTPs, 1.5 mmol/L $MgCl_2$, 0.8 μmol/L of each primer, 0.5 U *Taq* polymerase in a buffer of 50 mmol/L KCl, 10 mmol/L Tris-HCl, and 0.1% Triton X-100 (pH 9.0). The reactions were incubated at 95°C for 5 minutes, followed by 35 cycles of 95°C, 57°C, and 72°C for 1 minute each. Products were run on an ABI 377 sequencer and analyzed with Genescan and Genotyper software (Applied Biosystems, Foster City, CA). Allelic imbalance was recorded if the area under either allele peak was reduced in the tumor sample to less than 50% of its normal value with respect to the other allele.

Immunohistochemical Analysis of p53

Detection of accumulated p53 protein within tissues was performed on 4-μm paraffin-embedded sections, using an anti-human p53 monoclonal antibody (DO-7; Dako).[22] Antigen was detected after microwave antigen retrieval in 0.1 mol/L citrate buffer, and bound primary antibody was detected using horseradish peroxidase-labeled sheep anti-mouse antibody. Color was developed with diaminobenzidine substrate (Sigma), and sections were counterstained with hematoxylin.

Results

The pathological findings in this case fit well with the accepted definitions of HPS in terms of multiplicity, distribution, and size of the hyperplastic lesions.[3] Although most of the polyps in this case were hyperplastic in nature, one showed serrated adenomatous change, typified by a serrated architecture, with cells showing abundant eosinophilic cytoplasm, goblet cell depletion, and oval vesicular nuclei with prominent nucleoli and nuclear stratification (Figure 2B). No areas of moderate or severe dysplasia were seen in this lesion, and no adenomatous polyps of the usual type were seen within the colectomy specimen.

It was evident that the small carcinoma had arisen within a hyperplastic lesion, which surrounded it on all sides (Figure 1, A and B). There was also evidence of mild adenomatous change within this hyperplastic lesion (Figure 1, B and C), although this change was focal, as is often the case in such lesions.[19] While hyperplastic epithelium was continuous with some sections of the larger carcinoma, it is impossible to exclude the possibility of collision of this large and invasive tumor with a separate adjacent hyperplastic lesion. Neither carcinoma showed the phenotypic features of "serrated adenocarcinoma" reported by Jass in the setting of HPS.[8]

Immunohistochemical analysis of p53 showed strong accumulation of the protein within nuclei of the smaller tumor (Figure 1D). This was not seen in any of the cells of

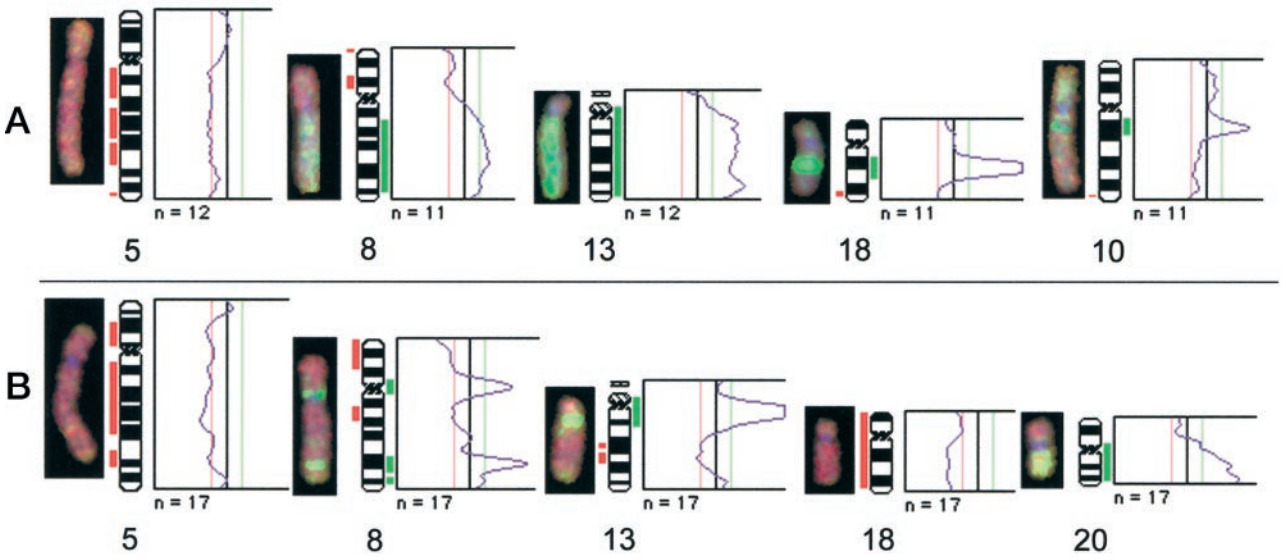

Figure 3. Representative chromosomes and CGH profiles in the large carcinoma (**A**) and small carcinoma (**B**). Chromosomal gains are indicated by green, and losses are shown in red.

the surrounding hyperplastic lesion, nor was it present in the serrated adenoma or the other four hyperplastic polyps examined. Interestingly, the larger tumor was also negative for nuclear p53 accumulation (not shown).

Comparative Genomic Hybridization Analysis

CGH was used to analyze molecular genetic abnormalities in DNA from both carcinomas, as well as from one of the hyperplastic polyps (HP1). No molecular genetic abnormalities were detected in the hyperplastic polyp. In contrast, the large carcinoma (T1) showed 11 chromosomal aberrations (five gains, six losses), and the small carcinoma (T2) showed 16 changes (nine gains, seven losses; Figures 3 and 4).

Although these changes were clearly not identical, several chromosomes were affected in both cancers, including 4, 5, 8, and 13, as shown schematically in Figure 4. The loss of 4q32-ter, 5q, and 18q12–21 seen in this study, as well as the high-level gains in 13q and 20q, have been reported frequently in sporadic colorectal carcinoma. Interestingly, the small carcinoma showed two regions of amplification at 8p11.2 and 8q23–24, and the large carcinoma showed a novel region of amplification at 10q21, as well as high-level amplification of 18q11.2–21.1.

Microsatellite Analysis

There was no evidence of microsatellite instability at any of the five loci examined, in either of the carcinomas, in the involved lymph node draining the larger tumor (LN), in the serrated adenoma (SA), or in any of five hyperplastic polyps examined (HP1–4, HP-T).

The D5S346 locus is found near the *APC* gene locus on chromosome 5, and the D17S938 locus is in close proximity to the *p53* gene. Loss of heterozygosity (LOH) at the D5S346 locus was observed in the large carcinoma as well as an involved draining lymph node, a result that was consistent with the loss of 5q observed in the primary tumor by CGH. However, LOH was not found at D5S346 in the small cancer, despite the fact that CGH had also demonstrated 5q loss in this tumor. As such, this may represent a homozygous deletion at this marker. The D17S938 locus showed LOH in both large and small carcinomas. In the case of the small cancer this loss was likely to be the result of an interstitial deletion, because CGH did not detect any abnormalities of chromosome 17. In contrast, CGH showed loss of chromosome 17 in the large tumor. Loss of heterozygosity at either the D5S346

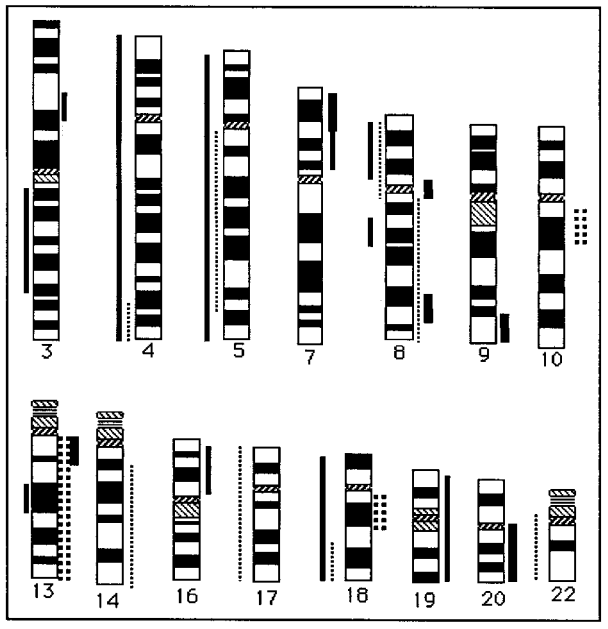

Figure 4. Summary of chromosomal changes observed in the large and small carcinomas. Gains are displayed to the right of the chromosomal ideogram, and losses are shown on the left. Changes in the small carcinoma are shown as solid lines and changes in the large carcinoma as dotted lines. Double lines mark regions affected by high-level gain or loss. Chromosomes that were normal in both lesions are not shown.

or D17S938 locus was not seen in any of the five hyperplastic polyps, including the lesion that gave rise to the cancer, or in the serrated adenoma.

Polymerase chain reaction using microsatellite markers was also used to further define the abnormalities detected on chromosomes 13q and 18q. In the smaller carcinoma, allelic imbalance was observed at the D18S49 locus (18q12.1–12.2), consistent with the loss of 18q found by CGH. However, the region of high level gain on 18q noted in the larger cancer was not reflected in allelic imbalance at this locus, possibly because D18S49 was not encompassed within the region of gain, or because the gain was "symmetrical." Allelic imbalance was also observed in both the large and small carcinomas, using markers D13S263 (13q14.1–21.1), D13S159 (13q22–31), and D13S174 (13q32–33), which is consistent with the 13q changes seen by CGH. In the case of the larger carcinoma, the pattern of change was likely to reflect the presence of multiple copies of chromosome 13, whereas the changes in the smaller tumor suggested more discrete regions of gain.

Discussion

The occurrence of two synchronous colonic carcinomas in the setting of hyperplastic polyposis, one of them at an early stage of invasion, is a rare event. It provides an opportunity to examine the critical biology of the disease, at the point of transformation from *in situ* dysplasia to invasive carcinoma.

We believe that the small carcinoma was very likely to have arisen within a pre-existing hyperplastic polyp that showed focal areas of adenomatous transformation. Although the possibility of a "collision tumor" cannot be totally excluded, the symmetry of the carcinoma, arising centrally within and completely surrounded by the hyperplastic lesion, makes this possibility remote. Furthermore, although hyperplastic patterns of reactivity are not uncommon in the transition from normal epithelium to tumor, the polypoid nature of the entire lesion and the presence of considerable hyperplastic tissue with a serrated architecture make this explanation also unlikely. Finally, the presence of areas within the surrounding lesion that showed evidence of early adenomatous transformation is an important indicator that this lesion was a consequence of neoplastic progression in a large hyperplastic polyp, of the type often seen in the hyperplastic polyposis syndrome.

It is interesting in this context that much of the putative precursor lesion maintained the morphology of a hyperplastic polyp. For althoughe tumor progression may have occurred within dysplastic foci, it seems that these foci did not have a significant growth advantage over the more usual hyperplastic elements and thus failed to dominate the lesion. This observation is in keeping with the common observation of mixed hyperplastic polyps, in which foci of serrated adenoma coexist with hyperplastic epithelium, yet fail to demonstrate an overwhelming clonal growth advantage within the lesion. An alternative explanation could be that there is an extremely rapid progression from serrated adenoma to invasive carcinoma, yet the finding of serrated adenomas without severe dysplasia is common in the hyperplastic polyposis syndrome and indeed was observed in this case.

The closer analysis of tissues in this case, through the combined approaches of comparative genomic hybridization, microsatellite analysis, and p53 immunohistochemistry, has allowed us to better understand the nature of the neoplastic progression in these lesions. Furthermore, this combined approach has allowed validation of data gained separately through each modality. Three points can be made on the basis of these results: that carcinoma in HPS develops through the pathway of chromosomal instability, that mutation of p53 is an early event in this process, and that microsatellite instability was not important in this case.

Carcinoma Progresses by Normal Pathways

In the carcinomas analyzed in this study, both the pattern and number of molecular cytogenetic abnormalities mirrored those described in a number of recent CGH analyses of the common type of CRC.[23–25] For instance, in a study of 45 sporadic colorectal cancers, De Angelis found that aneuploid tumors had a median of 9.0 chromosomal abnormalities, with high-level gains seen frequently in 8q, 13q, and 20.[24] Both cancers in this study clearly exhibited more than nine changes; high-level gains of chromosome 8 and 13 were seen in both lesions, and high-level gain of chromosome 20q was also present in the smaller carcinoma. This latter finding in particular has been well described.[23,25] The significance of these amplifications in tumor progression remains unknown. Interestingly, the finding of allelic imbalance at 13q in both tumors may serve as a useful marker in the more rapid evaluation of the presence of amplification and in the closer exploration of the biological significance of this phenomenon in colorectal carcinoma.

Mutation of p53 Is an Early Event in Malignant Progression

It is clear that the smaller carcinoma developed a mutation in the *p53* gene that was not present in the putative precursor lesion. This was demonstrated directly by immunohistochemistry and was consistent with the finding of LOH at the D17S398 locus. It is likely that this was a region of interstitial loss and hence would not be detected by CGH. Nevertheless, the finding of widespread chromosomal instability in the tumor, with a large number of discrete chromosomal abnormalities detected by CGH, further supports the hypothesis that malignant progression occurs as a result of *p53* inactivation and secondary chromosomal instability.

Althoughe the larger cancer showed widespread chromosomal instability, LOH at D17S398, and extensive loss of chromosome 17 by CGH, it was interesting that the immunohistochemistry for p53 was negative. The most likely explanation is that the remaining *p53* allele had a frameshift, chain-terminating or nonsense mutation that caused failure of protein expression, an event that under-

lies the imperfect correlation between immunohistochemical results and mutations in *p53*.[26]

On the other hand, we could find no evidence of *p53* mutation in any precursor lesions by immunohistochemistry and no LOH at D17S398 in five hyperplastic polyps and one serrated adenoma. Although *p53* mutations are rare in hyperplastic polyps,[27,28] there is considerable variation in the literature regarding the incidence of p53 positivity in serrated adenomas, with reports varying from 5% to 65%.[27,29] To some extent, this may simply reflect variation in the degree of dysplasia within the lesions of each series and, by default, the diagnostic criteria applied to the selection of cases. Extrapolation of data from this study suggests that lesions with high-grade dysplasia (carcinoma *in situ*) are likely to contain *p53* mutations, whereas serrated adenomas with only low-grade dysplasia will show no abnormalities.

In the common form of colorectal neoplasia, mutation of *p53* is known to be a critical event in the progression from adenoma to invasive carcinoma.[30] However, the reasons for the development of mutations at this point in the adenoma-carcinoma sequence are not clear. It has been suggested that the formation of an adenoma, with its disordered crypt architecture, allows the exposure of proliferating cells to fecal carcinogens.[3] This leads in some cases to *p53* mutations and subsequent neoplastic progression via impaired apoptosis and chromosomal instability. It is plausible that a similar mechanism may be at work in the hyperplastic polyp/serrated adenoma lesions typical of the hyperplastic polyposis syndrome.

The Nature of the Underlying Defect Remains Uncertain

This study has failed to elucidate the exact nature of the molecular lesions that underpin the HP syndrome. The lack of chromosomal abnormalities detectable by CGH in the precursor lesions is in sharp contrast to reports of chromosomal aberrations in adenomas[25,31] and suggests that the CGH approach is unlikely to throw further light on this issue.

We found no evidence of microsatellite instability in either the carcinomas or any of the precursor lesions examined, including hyperplastic polyps and a serrated adenoma. In fact, no instability was found at any locus, despite the use of both fresh tissue and carefully microdissected materials. This is in contrast to the findings of Jass, who showed low-level microsatellite instability in serrated adenomas in the setting of HPS and proposed that it may be an important event in the development of subsequent carcinomas.[15]

With regard to this discrepancy, we consider the possibility of gross contamination of tumor DNA by normal tissue to be an unlikely explanation for the absence of microsatellite instability. In fact, the relative purity of the tumor DNA is evidenced by our ability to demonstrate LOH at several alleles in both tumor samples and the correlation between these findings, CGH, and immunohistochemistry. The use of different microsatellite markers and, in particular, the use of tetranucleotide repeat markers and the MYCL markers favored by Jass may contribute to differences reported here.

The true biological significance of the microsatellite low-instability phenotype remains uncertain, and the definition of this phenomenon remains both arbitrary and subject to considerable operational flexibility. Clearly it is not a universal event in HPS or important in tumor progression in this case. However, it is possible that there is variability in the process of carcinogenesis within the hyperplastic polyposis syndrome, and indeed the syndrome may represent a phenotype with more than one genetic basis.

In summary, this case provides morphological and genetic evidence that carcinoma developing in the setting of hyperplastic polyposis can arise directly from a hyperplastic lesion. In this case, microsatellite instability was not important in either the development of the primary lesion or subsequent tumor progression. Rather, progression of the lesions involved loss of *p53* and the development of chromosomal instability, a pathway of neoplastic progression also seen in the common form of colorectal carcinoma.

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