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*Advances in Brief*

# The Identification of Monoclonality in Human Aberrant Crypt Foci[1]

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## Abstract

**Malignant neoplasms, including colon cancers, are thought to arise from a single initiated progenitor cell. Aberrant crypt foci (ACF) are putative precursors of at least some colon cancers. The pattern of X chromosomal inactivation, which is identified by the differential methylation of a site near a polymorphic CAG repeat in the androgen receptor gene, was used to determine the clonality status of 11 ACF from eight female patients. Ten of 11 ACF were found to be monoclonal aberrations. The eleventh ACF appeared monoclonal, but nonrandom inactivation of the X chromosome was also seen in normal crypts from this patient. These results clearly demonstrate that: (*a*) a high percentage of ACF lesions are neoplastic rather than hyperplastic; and (*b*) ACF are the earliest identified neoplastic lesions in the colon.**

## Introduction

Colorectal cancer is the second most frequent cause of cancer deaths in the United States (1); thus, it is imperative that methods of early detection be found. ACF[3] are lesions that are identified microscopically in colonic mucosa that appears normal by visual inspection. They are comprised of crypts that are microscopically elevated above the normal colonic mucosa, have thickened epithelia and altered luminal openings, and are clearly circumscribed from adjacent normal crypts (2). Because these lesions were first observed in the colons of rodents treated with colon-specific carcinogens and later observed at a higher incidence in the colons of patients with sporadic and inherited colon cancer as compared to those of patients without colon cancer (Ref. 2; reviewed in Ref. 3), it was hypothesized that ACF are precursors of colorectal cancer. Subsequent biochemical, genetic, and morphological studies (reviewed in Ref. 3; Ref. 4) have shown many similar alterations in colon tumors and ACF that further support the hypothesis that ACF may be the precursors of some colon tumors. It was proposed over 20 years ago that most tumors develop from a single initiated cell (5); since then, it has been shown that many human tumors are indeed monoclonal (6–8). It is not known what genetic alteration(s) occur to initiate those cells that are the progenitors of clonal populations. It could be the aberrant expression or structural alteration of the known oncogenes and tumor suppressor genes or of unknown gene(s). Identification of monoclonality in a putative precursor lesion would strongly link this lesion to neoplastic progression. We have determined the clonal composition of paraffin-embedded human ACF with various degrees of dysplasia by studying the differential methylation of a site in the first exon of the androgen receptor gene to determine the pattern of X chromosome inactivation (7–9). Our findings indicate that a high percentage of ACF are monoclonal, *i.e.*, neoplastic. More importantly, ACF can be used to study the mechanism of clonal evolution of colon cancer. They may provide a means of early detection.

## Materials and Methods

**Specimens.** ACF that were previously evaluated for the presence and degree of dysplasia (4) were used for this study. Briefly, ACF identified microscopically in grossly normal colonic mucosa were embedded in paraffin from which 5-$\mu$m-thick serial sections were cut. Twenty-five ACF in the previous study were from females. Two of these ACF were adjacent to each other and could not be microdissected separately with complete confidence; a third ACF, which was composed of only 15 crypts, lacked a sufficient number of sections to be evaluated. This left 22 ACF from 14 female patients with no more than 3 ACF/patient for this study.

**DNA Preparation.** DNA was isolated from serial paraffin sections in the following manner. The slides of the ACF were deparaffinized through xylenes and decreasing alcohols into water, stained for 2 min with hematoxylin or 1% methyl green, and stored in distilled water for 20–60 min before being dissected. Using a 1-$\mu$m needle (Fisher Scientific, Pittsburgh, PA), approximately five to eight crypts from six to eight sections of each ACF were scraped into 20 $\mu$l of digestion buffer [50 mM Tris (pH 8.5), 0.5% Tween 20, and 200 $\mu$g/ml proteinase K (Sigma, St. Louis, MO)] and incubated at 55°C for 3 h (10). Samples of normal colonic tissue that included stromal, muscle, and epithelial cells were scraped from the same slides as used for the ACF. Another set of six to eight sections was used for the microdissection of epithelial cells from five to eight normal crypts and their adjacent normal colonic tissue. An example of a microdissection of crypts is shown in Fig. 1. Proteinase K was inactivated with phenylmethylsulfonyl fluoride (Boehringer Mannheim, Indianapolis, IN) at a final concentration of 100 mM (11). This DNA solution (9–10 $\mu$l) was either mock-digested with the digestion buffer and no enzyme or digested with 60 units of *Hpa*II (40 units/ml; Boehringer Mannheim) in a total volume of 15 $\mu$l overnight at 37°C.

**PCR.** The sequences for the primers [which were obtained from Research Genetics (Huntsville, AL)] of the polymorphic region of the androgen receptor gene are as follows: forward, 5′-GCTGTGAAGGTTGCTGTTCCTCAT-3′; and reverse, 5′-TCCAGAATCTGTTCCAGAGCGTGC-3′ (9). For each PCR reaction, 4 $\mu$l of the DNA restriction digest were amplified with the following PCR mix in a total volume of 25 $\mu$l: 200 $\mu$M deoxynucleotide triphosphates (Life Technologies, Inc., Gaithersburg, MD), 1× Q solution (Qiagen, Chatsworth, CA), 0.8 $\mu$M unlabeled primer, and 0.16 $\mu$M of the other primer end-labeled with [$\gamma$-$^{33}$P]ATP. The end-labeling reaction was performed by incubating the following mixture at 37°C for 30–60 min: 5 $\mu$l (20 $\mu$M) primer; 5 $\mu$l of 5× labeling buffer (Life Technologies, Inc.); 5 $\mu$l of [$\gamma$-$^{33}$P]ATP (10 $\mu$Ci/$\mu$l; 4000 Ci/mmol; Amersham, Arlington Heights, IL); 1 $\mu$l of T4 polynucleotide kinase (Life Technologies, Inc.); and 9 $\mu$l of distilled $H_2O$ for a total volume of 25 $\mu$l. After an initial denaturation of the PCR reaction mixture at 95°C for 2 min, 1.25 units of Taq (Qiagen) in 1× PCR buffer (Qiagen) were added to the samples as a hot start. The samples were amplified for 35 cycles (PTC-100 thermocycler; MJ Research, Watertown, MA) under the following conditions (which were modified from Ref. 7): 15 s at 95°C; 30 s at 60°C; and 30 s at 72°C.

**Analysis of Clonality.** Amplified DNA (6 $\mu$l) was added to 4 $\mu$l of 40% formamide loading buffer; 3 $\mu$l of this mixture were loaded onto a 6% polyacrylamide gel and run at 55 W for 1.5–3 h. The gel was fixed for 10–15 min in 5% acetic acid and 10% methanol, dried, and exposed to X-ray film

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[3] The abbreviations used are: ACF, aberrant crypt foci; $R_{AA}$, allele amplification ratio; $R_{AI(N)}$, allele inactivation ratio for the normal tissue; $R_{AI(E)}$, allele inactivation ratio for the experimental tissue.



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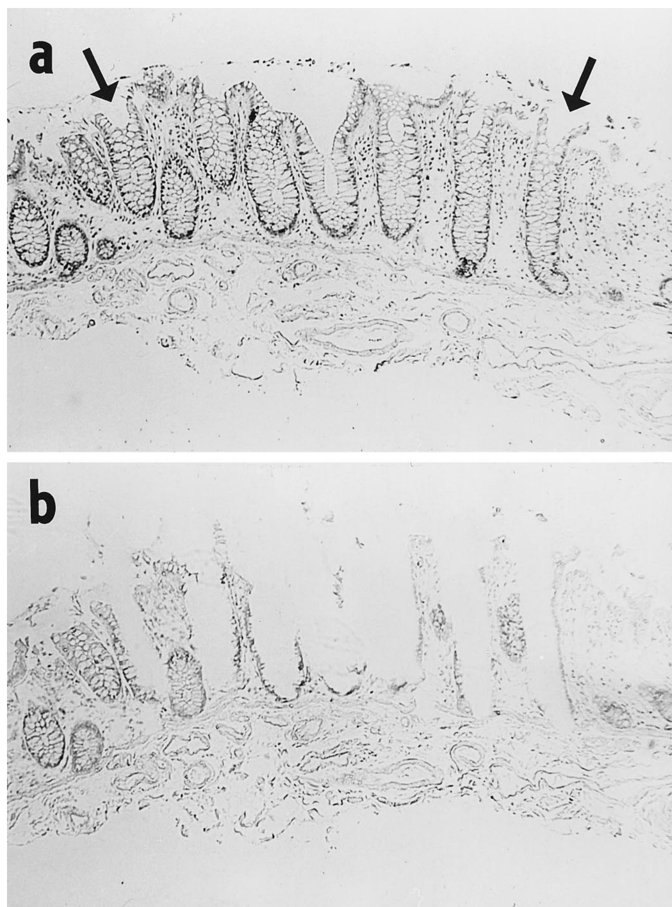


Fig. 1. Microdissection of crypts from a paraffin section from patient 179. The section (*a*) was stained with H&E, and the microdissected section (*b*) was stained with 1% methyl green before microdissection. *Arrows*, the boundaries of the microdissection; approximately eight crypts were microdissected in *b*. *a* and *b*, ×50.

(Fuji X-OMAT; Fisher Scientific) and a Kodak TranScreen LE intensifying screen (Eastman Kodak, New Haven, CT) at −70°C for 19–21 h. Densitometry readings were performed on the autoradiograms with the 420 oe scanner (42 × 42-μm resolution with a white filter and an absorbance setting of 3.0) and QS30 software, version 2.5 (PDI, Huntington, NY). Each lane was read separately, and the intensities of the bands in each lane were measured as a percentage of the total intensity of the entire lane delimited. Ratios were then calculated in the following manner (8). The $R_{AA}$ for each sample before digestion with *Hpa*II was calculated by dividing the relative percentage reading of the high molecular weight band by the relative percentage reading of the low molecular weight band; a ratio equal to 1 would demonstrate equal amplification of both alleles. For an experiment to be included, the $R_{AA}$ had to be equal to or greater than 0.6. The $R_{AI(N)}$ and $R_{AI(E)}$ were calculated by first obtaining the ratios of the high and low molecular weight bands for the (*a*) undigested samples and (*b*) the same samples after digestion with *Hpa*II. Next, these ratios (*a* and *b*) were divided such that the larger ratio was the denominator, *i.e.*, $R_{AI(N)}$ or $R_{AI(E)} < 1$. The $R_{AI(N)}$ for the normal tissue had to be near 1 to allow the results from the corresponding experimental tissues to be included in the study. If $R_{AI(E)} < 0.4$, the sample was considered monoclonal; if $R_{AI(E)} > 0.4$, the sample was considered polyclonal (8).

## Results

Clonality was demonstrated by determining the pattern of X inactivation with PCR amplification of a region near a highly polymorphic trinucleotide repeat in the first exon of the androgen receptor gene on the X chromosome after digestion with a methylation-sensitive enzyme, *Hpa*II, which is specific for that site (9). In normal females, either the paternal or maternal X chromosome is inactivated randomly by methylation, leading to a mosaic pattern of X inactivation in normal tissue. However, a monoclonal population of cells arising from a single progenitor cell would have the same inactivated X chromosome in all cells, and incubation with *Hpa*II would lead to the digestion of the same active X chromosome. Before digestion with *Hpa*II, both monoclonal tissue and normal tissue DNA would yield two alleles after PCR amplification. After digestion with *Hpa*II, the two alleles of the normal tissue DNA would be diminished by roughly the same amount, whereas only one allele of monoclonal tissue DNA would be expected to be present.

PCR studies were carried out on 22 ACF from 14 patients; four ACF from two patients were eliminated because the androgen receptor alleles from these patients appeared to be homozygous, *i.e.*, were noninformative. This left 18 ACF from 12 female patients to be analyzed for clonality in this study. PCR amplification of the DNA from both the normal tissue and the ACF before and after digestion with *Hpa*II was successful for 11 (Table 1; Fig. 2) of the 18 ACF. The $R_{AA}$ of the high molecular weight allele to the low molecular weight allele for normal colonic tissue, which includes stromal cells and epithelial cells, ranged between 0.65 and 1.39, with a mean of 0.90 ± 0.20 (± SD; Table 1); this indicated approximately equal amplification of both androgen receptor alleles in each normal tissue sample analyzed for each patient, with a slight overamplification of the lower molecular weight allele as reported previously (8). The $R_{AI(N)}$ of this same tissue was close to 1 (0.81 ± 0.11, mean ± SD); this indicated approximate random inactivation of X chromosomes in the normal tissues analyzed. Duplicate analyses of each of the 11 ACF indicated a monoclonal population in all 11 ACF because the $R_{AI(E)}$ was < 0.4 (Table 1). The ACF ranged in size from 25 crypts to 225 crypts, included 7 ACF with mild dysplasia and 4 ACF with only atypia, and were located throughout the colon (5 ACF from the ascending colon, 1 ACF from the transverse colon, and 5 ACF from the distal colon). Two different ACF from patient 1429 showed inactivation of different androgen receptor alleles, whereas two ACF from patient 1900 showed inactivation of the same allele (Fig. 2). Epithelial cells that were microdissected from five to eight adjacent normal crypts from the same patients demonstrated polyclonal populations in seven of the eight patients (Table 2; Fig. 2). Two groups of eight normal crypts from two different locations from patient 1897 indicated monoclonal populations of cells with the same allele inactivated in each group of normal crypts. The monoclonal population of cells observed in the ACF from patient 1897, however, had the other allele inactivated (data not shown).

Seven of the 18 ACF were eliminated from this study because inconsistent results were obtained from multiple PCR analyses of the same tissues. As noted above, we required approximately equal amplification of both alleles in the normal control tissue before and after digestion with *Hpa*II. In addition, we required approximately equal amplification of both alleles for the ACF sample before digestion with *Hpa*II and detectable amplification of the sample after digestion with *Hpa*II. If any of these conditions were not met, the data for that ACF were considered invalid. Whereas some analyses of the 11 ACF included in Table 1 were inadequate for interpretation, we were able to obtain repeatable results when the above conditions were met. For the seven ACF that were omitted, repeatable results were not obtained even after multiple analyses.

## Discussion

Our finding that at least 10 of 11 ACF from eight female patients exhibit nonrandom X inactivation that is not seen in morphologically normal crypts from the same patients indicates that a high percentage of ACF are monoclonal and thus neoplastic. Previously, we reported



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Table 1 *Analysis of ACF by densitometry after PCR amplification of the androgen receptor*

| Patient no. | ACF (trial no.) | Size[a] | No. of crypts dissected[b] | $R_{AA}$[c] | $R_{AI(N)}$[d] | $R_{AI(E)}$[e] | Location of ACF | Pathology of ACF |
|---|---|---|---|---|---|---|---|---|
| 1897 | A (1) | 110 | 6 | 0.81 | 0.85 | 0 | Ascending colon | Mild dysplasia |
| | A (2) | | 6 | 0.89 | 0.74 | 0.18 | | |
| 2101 | A (1) | 27 | 7 | 0.92 | 0.80 | 0.09 | Ascending colon | Mild dysplasia |
| | A (2) | | 7 | 0.88 | 0.75 | 0.30 | | |
| 221 | A (1) | 72 | 8 | 1.01 | 0.70 | 0 | Ascending colon | Mild dysplasia |
| | A (2) | | 8 | 1.05 | 0.71 | 0 | | |
| | B (1) | 225 | 8 | | | 0 | Ascending colon | Atypia |
| | B (2) | | 8 | | | 0 | | |
| 179 | A (1) | 64 | 7 | 0.73 | 0.86 | 0.04 | Ascending colon | Atypia |
| | A (2) | | 7 | 1.11 | 0.79 | 0 | | |
| 1807 | D (1) | 19 | 5 | 0.90 | 0.96 | 0.11 | Transverse colon | Mild dysplasia |
| | D (2) | | 5 | 1.39 | 0.60 | 0 | | |
| 1429 | A (1) | 91 | 8 | 0.65 | 0.91 | 0.06 | Sigmoid | Atypia |
| | A (2) | | 8 | 0.70 | 0.80 | 0.13 | | |
| | B (1) | 200 | 8 | | | 0.24 | Sigmoid | Atypia |
| | B (2) | | 8 | | | 0.19 | | |
| 638 | B (1) | 50 | 5 | 1.09 | 1.00 | 0.27 | Rectum | Mild dysplasia |
| | B (2) | | 5 | 0.73 | 0.87 | 0 | | |
| 1900 | A (1) | 25 | 7 | 0.76 | 0.87 | 0.11 | Rectum | Mild dysplasia |
| | A (2) | | 7 | 0.73 | 0.70 | 0 | | |
| | B (1) | 41 | 7 | | | 0.13 | Rectum | Mild dysplasia |
| | B (2) | | 7 | | | 0 | | |
| Total | | | | | | | | |
| 8 | 11 | | 6.8[f] | 0.90 | 0.81 | 0.08 | | |
| | | | ±1.1 | ±0.20 | ±0.11 | ±0.10 | | |

[a] Number of crypts per ACF.
[b] Number of crypts microdissected within an ACF.
[c] Allele amplification ratio for normal colonic tissue (mixture of stromal and epithelial cells).
[d] Allele inactivation ratio after digestion with *Hpa*II for normal colonic tissue (mixture of stromal and epithelial cells).
[e] Allele inactivation ratio after digestion with *Hpa*II for epithelial cells in five to eight crypts from an ACF.
[f] Mean ± SD.

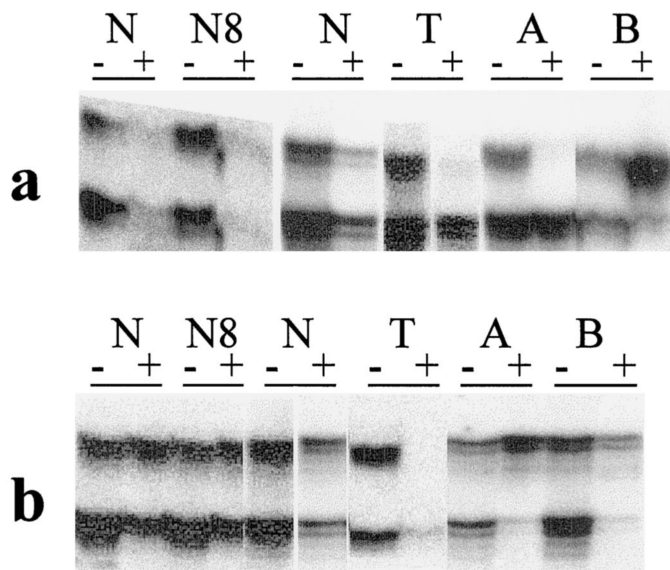


Fig. 2. Autoradiograms of PAGE of PCR products for the androgen receptor gene with DNA from four ACF along with the corresponding normal controls and colorectal tumors from two different patients. *a*, samples from patient 1429; *b*, samples from patient 1900. *Lanes N*, normal colonic tissue including stromal, muscle, and epithelial cells; *Lanes N8*, epithelial cells from eight normal crypts; *Lanes T*, epithelial cells from the tumor; *Lanes A*, epithelial cells from ACF-A; *Lanes B*, epithelial cells from ACF-B; −, mock digested; +, digested with *Hpa*II. The first four lanes correspond to data in Table 2; the next eight lanes correspond to data in Table 1. A different set of sections was analyzed for the data in the two tables.

that 54% of human ACF exhibit some degree of dysplasia, including carcinoma *in situ* (4). Of the 10 ACF that were demonstrated to be monoclonal, no apparent correlation was found between clonality and the presence and/or degree of dysplasia in the ACF, *i.e.*, both ACF with and without dysplasia were found to be monoclonal (Table 1). However, this study examined only 11 ACF, and a greater sample size needs to be analyzed to confirm this finding. If results from future studies agree with the high percentage of monoclonal ACF found in this study and with the demonstration of monoclonality in ACF without dysplasia, they would support the hypothesis that the event(s) that results in monoclonality precedes dysplastic changes of precancerous colonic epithelium. In a recent model of colon tumorigenesis (12), the earliest stage of colon cancer proposed is dysplastic ACF,

Table 2 *Analysis of normal crypts by densitometry after PCR amplification of the androgen receptor*

| Patient no. | Trial no. | No. of crypts dissected[a] | $R_{AA}$[b] | $R_{AI(N)}$[c] | $R_{AI(E)}$[d] |
|---|---|---|---|---|---|
| 1897 | 1 | 7 | 0.60 | 0.91 | 0 |
| | 2 | 8 | 0.91 | 0.88 | 0 |
| 2101 | 1 | 8 | 0.90 | 0.83 | 0.79 |
| | 2 | 8 | 0.82 | 0.75 | 0.77 |
| 221 | 1 | 8 | 1.00 | 0.81 | 0.80 |
| | 2 | 8 | 1.00 | 0.79 | 0.64 |
| 179 | 1 | 8 | 0.78 | 0.76 | 0.81 |
| | 2 | 8 | 1.06 | 0.88 | 0.98 |
| 1807 | 1 | 5 | 1.12 | 0.82 | 0.45 |
| | 2 | 5 | 0.88 | 0.98 | 0.80 |
| 1429 | 1 | 8 | 0.96 | 0.63 | 0.86 |
| | 2 | 8 | 0.63 | 0.79 | 0.95 |
| 638 | 1 | 7 | 0.85 | 0.96 | 0.95 |
| | 2 | 7 | 0.86 | 0.91 | 0.70 |
| 1900 | 1 | 8 | 0.60 | 0.60 | 0.72 |
| | 2 | 8 | 0.70 | 0.69 | 0.78 |
| Total | | | | | |
| 8 | | 7.4[e] | 0.85 | 0.81 | 0.79[f] |
| | | ±1.0 | ±0.16 | ±0.11 | ±0.14 |

[a] Number of normal crypts microdissected.
[b] Allele amplification ratio for normal colonic tissue (mixture of stromal and epithelial cells).
[c] Allele inactivation ratio after digestion with *Hpa*II for normal colonic tissue (mixture of stromal and epithelial cells).
[d] Allele inactivation ratio after digestion with *Hpa*II for epithelial cells in five to eight normal crypts.
[e] Mean ± SD.
[f] Mean ± SD of samples that appear polyclonal, *i.e.*, excluding those from patient 1897.



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which is caused by a mutation in the *APC* gene. The finding of monoclonality in ACF without dysplasia identifies ACF as the earliest manifestation of neoplasia in the colon. Interestingly, two ACF from the same patient showed different X inactivation patterns (Fig. 2); this confirms that individual ACF arise from independent initiation events.

Normal crypts in both mice (13) and humans (14) are monoclonal populations that arise from single stem cells, whereas patches of 6–10 normal crypts are polyclonal populations (15). Because patches of one to four crypts appear to be monoclonal in normal mouse colon (15), at least five crypts from each ACF were microdissected. An equivalent number of normal crypts from each patient were microdissected and analyzed by the PCR assay. In seven of the eight patients in this study, microdissection of equivalent numbers of adjacent normal crypts from the normal mucosa and aberrant crypts within an ACF led to patterns of random X inactivation in normal crypts and nonrandom X inactivation in ACF. The normal crypts microdissected from one patient exhibited a nonrandom pattern of X inactivation. However, the pattern of X inactivation of the eight normal crypts from two different locations was similar but differed from that of the ACF from this patient. These results are consistent with monoclonality of the ACF in a manner similar to that reported for a tumor specimen by Mashal *et al.* (7).

With antibodies to detect H-2 antigens as markers of parental origin and cellular genotype, dysplastic foci in the colons of mice treated with a colon-specific carcinogen were shown to be monoclonal (16). Human colorectal cancers have been demonstrated to be monoclonal (6), although there are reports that some colonic adenomas in both humans (17, 18) and mice (19) are polyclonal in origin. All of the reports of polyclonality in some colon tumors are from studies with humans or mice that inherited a mutant copy of the *APC* gene and thus may not be applicable to sporadic colon cancer. The presence of polyclonality in these colon tumors that arise in a background of an inherited mutant *APC* allele may be evidence for a dominant effect of the mutation as suggested by others (20).

The finding of heterozygosity for the androgen receptor in 12 of 14 (87%) patients from whom ACF were analyzed is in the expected range for this assay (7). A total of 7 of the 18 (39%) ACF that were informative with the androgen receptor assay were eliminated from this study because of persistently inconclusive and/or uninterpretable results. Lucas *et al.* (8) had unsuccessful results from 4 of 12 (33%) cases, and Mashal *et al.* (7) found skewing of the band intensities from normal tissues after digestion with *Hha*I in 3 of 13 (23%) cases; they discuss various reasons for this skewing, including nonrandom inactivation of the X chromosome. Mashal *et al.* (7) comment that "not all samples produced efficient amplification of DNA if the Chelex step was eliminated." Because our samples of DNA (even those from multiple sections of ACF) contained less DNA than those from the single 10-$\mu$m sections used by Mashal *et al.* (7), we were unable to carry out the recommended purification with Chelex. The inconclusive results for seven ACF are most likely due to technical problems that may include overfixation of the tissue, loss of the tissue sample during microdissection, the presence of contaminants in the tissue, or incomplete digestion by the restriction enzyme *Hpa*II. In addition, DNA extracted from archival paraffin blocks tends to be partially degraded as well as contaminated by blood or metals; this frequently leads to biased amplification of alleles and even a variation in the relative intensity of human androgen receptor products from different analyses of the same DNA samples (21).

Our finding of monoclonality in ACF, including those without dysplasia, puts ACF at the earliest identified stage of neoplasia in the colon. Additional studies with larger sample sizes of ACF are needed to conclusively prove this hypothesis.

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## References


1. Landis, S. H., Murray, T., Bolden, S., and Wingo, P. A. Cancer statistics, 1998. CA Cancer J. Clin., *48:* 6–29, 1998.
2. Bird, R. P. Observation and quantification of aberrant crypts in the murine colon treated with a colon carcinogen: preliminary findings. Cancer Lett., *37:* 147–151, 1987.
3. Pretlow, T. P., and Pretlow, T. G. Putative preneoplastic changes identified by enzyme-histochemical and immunohistochemical techniques. J. Histochem. Cytochem., *46:* 577–583, 1998.
4. Siu, I-M., Pretlow, T. G., Amini, S. B., and Pretlow, T. P. Identification of dysplasia in human colonic aberrant crypt foci. Am. J. Pathol., *150:* 1805–1813, 1997.
5. Fialkow, P. J. The origin and development of human tumors studied with cell markers. N. Engl. J. Med., *291:* 26–35, 1974.
6. Fearon, E. R., Hamilton, S. R., and Vogelstein, B. Clonal analysis of human colorectal tumors. Science (Washington DC), *238:* 193–197, 1987.
7. Mashal, R. D., Lester, S. C., and Sklar, J. Clonal analysis by study of X chromosome inactivation in formalin-fixed paraffin-embedded tissue. Cancer Res., *53:* 4676–4679, 1993.
8. Lucas, D. R., Shroyer, K. R., McCarthy, P. J., Markham, N. E., Fujita, M., and Enomoto, T. E. Desmoid tumor is a clonal cellular proliferation: PCR amplification of HUMARA for analysis of patterns of X-chromosome inactivation. Am. J. Surg. Pathol., *21:* 306–311, 1997.
9. Allen, R. C., Zoghbi, H. Y., Moseley, A. B., Rosenblatt, H. M., and Belmont, J. W. Methylation of *HpaII* and *HhaI* sites near the polymorphic CAG repeat in the human androgen-receptor gene correlates with X chromosome inactivation. Am. J. Hum. Genet., *51:* 1229–1239, 1992.
10. Markowitz, S., Hines, J. D., Lutterbaugh, J., Myeroff, L., Mackay, W., Gordon, N., Rustum, Y., Luna, E., and Kleinerman, J. Mutant K-*ras* oncogenes in colon cancers do not predict patient's chemotherapy response or survival. Clin. Cancer Res., *1:* 441–445, 1995.
11. Tsai, Y. C., Simoneau, A. R., Spruck, C. H., III, Nichols, P. W., Steven, K., Buckley, J. D., and Jones, P. A. Mosaicism in human epithelium: macroscopic monoclonal patches cover the urothelium. J. Urol., *153:* 1697–1700, 1995.
12. Kinzler, K. W., and Vogelstein, B. Lessons from hereditary colorectal cancer. Cell, *87:* 159–170, 1996.
13. Ponder, B. A. J., Schmidt, G. H., Wilkinson, M. M., Wood, M. J., Monk, M., and Reid, A. Derivation of mouse intestinal crypts from single progenitor cells. Nature (Lond.), *313:* 689–691, 1985.
14. Endo, Y., Sugimura, H., and Kino, I. Monoclonality of normal human colonic crypts. Pathol. Int., *45:* 602–604, 1995.
15. Gould, K. A., and Dove, W. F. Localized gene action controlling intestinal neoplasia in mice. Proc. Natl. Acad. Sci. USA, *94:* 5848–5853, 1997.
16. Ponder, B. A. J., and Wilkinson, M. M. Direct examination of the clonality of carcinogen-induced colonic epithelial dysplasia in chimeric mice. J. Natl. Cancer Inst., *77:* 967–976, 1986.
17. Novelli, M. R., Williamson, J. A., Tomlinson, I. P. M., Elia, G., Hodgson, S. V., Talbot, I. C., Bodmer, W. F., and Wright, N. A. Polyclonal origin of colonic adenomas in an XO/XY patient with FAP. Science (Washington DC), *272:* 1187–1190, 1996.
18. Bjerknes, M., Cheng, H., Kim, H., Schnitzler, M., and Gallinger, S. Clonality of dysplastic epithelium in colorectal adenomas from familial adenomatous polyposis patients. Cancer Res., *57:* 355–361, 1997.
19. Merritt, A. J., Gould, K. A., and Dove, W. F. Polyclonal structure of intestinal adenomas in *Apc*$^{\mathrm{Min}/+}$ mice with concomitant loss of *Apc*$^{+}$ from all tumor lineages. Proc. Natl. Acad. Sci. USA, *94:* 13927–13931, 1997.
20. Mahmoud, N. N., Boolbol, S. K., Bilinski, R. T., Martucci, C., Chadburn, A., and Bertagnolli, M. M. *Apc* gene mutation is associated with a dominant-negative effect upon intestinal cell migration. Cancer Res., *57:* 5045–5050, 1997.
21. Mutter, G. L., and Boynton, K. A. PCR bias in amplification of androgen receptor alleles, a trinucleotide repeat marker used in clonality studies. Nucleic Acids Res., *23:* 1411–1418, 1995.




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