The Incidence of Aberrant Crypt Foci and Colonic Carcinoma in Dimethylhydrazine-treated Rats Varies in a Site-specific Manner and Depends on Tumor Histology

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ABSTRACT

In an attempt to demonstrate the relationship between aberrant crypt foci (ACF) and subsequent colonic neoplasms, we investigated the distribution of ACF in the dimethylhydrazine (DMH) model of colonic carcinogenesis in the rat. DMH was given to male Wistar rats by s.c. injection in a dosage of 15 mg/kg body weight once a week for 19 weeks. As a result, eight poorly differentiated, mucin-secreting carcinomas, two well-differentiated tubular adenocarcinomas, and four adenomas developed in 35 rats autopsied at 24 weeks after the first injection of DMH. The location of each type of tumor was site specific. Poorly differentiated, mucin-secreting carcinomas, two well-differentiated tubular adenocarcinomas, and four adenomas developed in the distal colon; the mean location of these lesions was 17.6 ± 3.8% (SE; range, 0–39%) of the length of the colon. Well-differentiated tubular adenocarcinomas and adenomas developed in the distal colon; the mean location of these lesions was 76.7% ± 4.9% (SE; range, 60–90%) of the length of the colon. There was a mean number of 276 ± 29 (SE) ACF per colon; these were present at between 40 and 90% of the colonic length, peaking at 70%. We conclude that ACF are marker lesions for colonic neoplasms, but only in the distal colon where tumors follow the adenoma-carcinoma sequence; this is not so for the proximal colon, where poorly differentiated, mucin-secreting carcinomas are found. These findings suggest that the latter tumors well may arise de novo and indicate that studies that attempt to correlate ACF with subsequent tumor formation must take cognizance, not only of the site, but also of the tumor type.

INTRODUCTION

The adenoma-carcinoma sequence has long been a central dogma that has explained many facets of colorectal carcinogenesis, and more recently, sequential genetic models have attempted to define the temporal sequence of morphological changes in the development from adenoma to carcinoma (1–4). The appearance of ACF has been strongly advocated as an intermediate marker of incipient neoplastic change during the process of colorectal carcinogenesis in the important DMH experimental model in rats (5–7) and humans, especially in familial adenomatous polyposis (8–10). However, confusion has been generated by recent reports that ACF and colon carcinomas developed in different sites in the colon after DMH treatment (11) and that ACF and adenoma incidences were not correlated in various strains of mice (12), with the resultant conclusion that ACF were consequently not a satisfactory preneoplastic marker. Previous observations from our group (13, 14), however, that the locations at which DMH-induced colorectal carcinomas developed varied depending on their histology, have largely been ignored in this context. We, therefore, hypothesized that ACF are preneoplastic markers only for a subset of carcinomas; if this were so, then general conclusions about the preneoplastic marker status of ACF would be inadvisable unless the associated histology of the related colon carcinomas are considered. We, therefore, investigated the relationship among ACF, site, and tumor histology in the rat DMH model.

MATERIALS AND METHODS

Rat DMH Model. Fifty male Wistar rats, ages between 8 and 10 weeks and weighing about 275 g, were studied. Animals were maintained in a temperature-controlled room with a 12-h light-dark cycle. Food and water were available ad libitum, and all animals were weighed weekly.

All animal experiments were approved by the Imperial Cancer Research Fund Animal Ethical Committee. DMH (Sigma D4137) was dissolved immediately before use in 1 mM EDTA and brought to pH 6.5.

Rats were given 19 weekly s.c. injections of either DMH in a dosage of 15 mg/kg body weight (n = 35) or the same volume of vehicle (n = 15; 1 ml/kg body weight). Rats were killed by cervical dislocation at 24 weeks after the first injection of DMH.

Tissue Preparation. The abdomen was opened, and the entire colon was removed. The colons were opened longitudinally, rinsed with ice-cold saline, blotted on plastic-coated paper, and fixed in Carnoy’s fluid for 3 h; the colons were transferred to 70% ethanol for storage after carefully recording the presence of any tumors.

ACF Measurement. Methylene blue was made up as a 0.1% solution in PBS. Open and laid-out colons were soaked in the methylene blue solution for 3 min. ACF were identified by their protuberant, large, and smooth-contoured crypt openings (5, 6) and carefully scored in both number and size along the whole colon, under the ×10 magnification of a compound microscope.

ACF were only measured in 14 rats because 12 rats were excluded as they had tumors, to avoid any bias of the results caused by altered physiology or any other factors due to the presence of tumors. Furthermore, nine rats did not have an intact mucosal surface and could not be scored. Colons were divided into 10 parts, depending on the distance from the caecum, recorded as 0% (caecum), 10% (between 1 and 10%), 20% (between 11 and 20%), 30, 40, 50, 60, 70, 80, 90, and 100% of the colonic length. During the scoring of ACF, microscopic adenomas or carcinomas were also detected and submitted for histological examination.

RESULTS

There was no mortality in either control or DMH-treated rats. Body weight changes showed no difference between the control and the DMH-treated groups. In total, 14 tumors developed in 12 rats among the 35 DMH-treated rats. All 35 rats had ACF (Table 1; Fig. 1). Eight rats developed macroscopically visible "napkin-ring"-shaped tumors, measuring between 0.5 and 1 cm in length, one in the caecum and seven in the proximal colon. Histology showed that all of these were poorly differentiated, mucin-secreting carcinomas (Fig. 2a). After methylene blue staining, six microscopic tumors became visible (Fig. 2b); histology showed four to be tubular adenomas (Fig. 2c) and two to be well-differentiated tubular adenocarcinomas, resembling adenomas but with invasion through the muscularis mucosae. All these lesions were found in the distal colon, beyond the 60% point (Table 1; Fig. 1).

A mean of 276 ACF were found per colon (Table 1). Few ACF...
Table 1 The distribution of various tumors and ACF along the colon

The histopathological classification of the tumors induced by DMH was that proposed by Sunter et al. (13, 14). Group III carcinomas are defined as those tumors that are poorly differentiated, mucin-secreting adenocarcinomas with numerous signet-ring cells (Fig. 2a). Adenomas are defined as tubular or tubulo-villous lesions that do not show infiltration through the muscularis mucosae (Fig. 2c), whereas group I carcinomas are similar to the adenomas but show unequivocal evidence of invasion of well-differentiated adenocarcinoma through the muscularis mucosae. All of these lesions are well illustrated by Sunter et al. (13, 14).

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*a Number, total number of lesions.

were seen between the proximal 10 and 30% of the colon; 3 ACF (in 1 of 14 animals) in the proximal 10%, 1 ACF (in 1 of 14 animals) in the proximal 20%, and 7 ACF (in 3 of 14 animals) in the proximal 30% of the colon, but the number increased markedly at this point, with 91 ACF (in 11 of 14 animals) at 40%, 455 ACF (in 14 of 14 animals) at 50%, 1035 ACF (in 14 of 14 animals) at 60%, 1227 ACF (in 14 of 14 animals) at 70%, and 814 ACF (in 14 of 14 animals) at 80%, before falling in number as the distal colon and rectum were reached; 238 ACF (in 12 of 14 animals) were seen at 90% and 14 ACF (in 4 of 14 animals) at 100%. No ACF developed in the control rats.

Some ACF show some cystic features, as is seen in nascent adenomas in the mouse intestine (Fig. 3) in which an allele of the Apc gene is mutant (15, 16).

DISCUSSION

The results suggest that chemically induced carcinogenesis in the rat colon follows two distinct pathways: what might be called the de novo sequence, occurring without passing through an obligatory stage of ACF and leading to the development of poorly differentiated, mucin-secreting carcinomas in the proximal colon, whereas elsewhere the histogenesis follows the aberrant crypt foci-adenoma-carcinoma sequence, in the mid and distal colon. These two different pathways may simply reflect biological differences between these parts of the colon or might represent two separate pathways of carcinogenesis; whatever the nature of the difference, our findings suggest that ACF are an intermediate stage only for the better-differentiated tumors found in the distal colon and not for the poorly differentiated, mucin-secreting carcinomas of the proximal colon.

Certainly in the human, the adenoma-carcinoma sequence has been generally accepted. The evidence for this has been reviewed on many occasions (3, 4); of course in modern models of multistage colonic carcinogenesis, lesions accumulate mutations in several key genes between the stages of adenoma and carcinoma (1, 2). Additionally, in the human, most adenomas and carcinomas are known to occur in the left colon in both sporadic cancer and familial adenomatous polyposis patients (17). However, there have been considerable differences in opinion as to whether DMH-induced carcinogenesis in rodents follows this classical adenoma-carcinoma sequence, with some authors denying any adenoma stage prior to the development of carcinoma (18, 19) and others insisting and demonstrating unequivocally that such a stage does occur (20–22). However, it is regrettable that many authors have largely ignored the seminal work of Sunter et al. (13, 14), who classified the lesions that develop in the rat colon after DMH treatment into several types: adenomas and group I carcinomas, invasive adenocarcinomas where it was clear from the presence of remaining adenomatous tissue that the invasive carcinoma had developed from a preexisting adenoma; group II carcinomas, moderately...
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Thus, any consideration of histogenesis in the rat DMH model must perforce include consideration of the colonic site. This point is well illustrated by the work of Cameron et al. (11), who reported that no ACF were found in the proximal colon at 1 or 5 weeks after DMH, at a site where carcinomas eventually occur; they concluded that the carcinomas that form in the proximal colon seldom if ever arise from an ACF, and that consequently, the location, number, and size of the ACF that occur early after DMH exposure do not correlate with the location or predict the incidence of carcinomas that eventually form in the colon. However, it is clear that these authors did not classify the tumors appropriately, according to the classification of Sunter et al. (13). We would predict that the proximal tumors arising in this study were of the undifferentiated, mucin-secreting variety, thus explaining the apparent discrepancies in the study of Cameron et al. (11).

Morphologically distinguishable ACF have long been proposed as intermediate stage markers for colonic cancer, and as such has been used in many studies as an early index of neoplastic induction (5, 6). Moreover, genetic studies have shown that ACF have a high incidence of K-ras mutations, equaling, or even higher than, the rate found in adenomas (23–27) and genomic instability (in oligo A or microsatellite loci; Refs. 28 and 29). ACF have been reported to occur more frequently in familial adenomatous polyposis patients than in patients with sporadic cancer patients and also are found more often in the left colon (8–10). It is, therefore, of considerable import that a recent study (11) showed that ACF and colon cancer developed in different sites of colon in DMH-treated mice, implying that ACF and colonic neoplasms are not sequentially related.

ACF are a heterogeneous group both from a pathological and genetic basis (30); they have been classified on their histopathological differentiations and adenocarcinomas where no adenomatous tissue remains; and group III carcinomas, poorly differentiated, mucin-secreting carcinomas with numerous signet-ring cells. Group III carcinomas were confined to the proximal colon and remained constant in incidence from 24 to 33 weeks after DMH, whereas the incidence of groups I and II carcinomas increased with time. Sunter et al. (13, 14), therefore, concluded that: (a) the distal group I and II carcinomas developed from adenomas, following the classical adenoma-carcinoma sequence; and (b) group III carcinomas formed a distinct histogenetic subgroup. Sunter et al. (13, 14) provide clear illustrations of these four groups of lesions induced by DMH.

Fig. 2. a, a group III carcinoma as classified by Sunter et al. (Res. 13 and 14; a poorly differentiated, infiltrating, mucin-secreting carcinoma) found in the rat proximal colon (H&E, X250). b, an adenoma (asterisk) on the surface mucosa of unsectioned rat distal colon, stained with methylene blue (X40), which also shows several ACF (open arrows) in the surrounding mucosa. c, H&E of adenoma (X200).

Fig. 3. ACF showing cystic features (H&E, X250), similar to a nascent adenoma in the mice heterozygous mutant Apc gene, Apc^{Min+}.
appearances as of normal appearance (1%), as hyperplastic (65%), as stage 1 (characterized by the extension of the proliferative compartment to the crypt surface but with no change in the major proliferation compartment (24%), and as true adenomas (10%; Ref. 31). If ACF are indeed a heterogeneous entity, they could conceivably develop independently rather than differ in appearance over a time sequence. An important additional study would be to relate these significant morphological and kinetic features both to time and to site within the colon.

From the results described in this report, we propose, however, that conclusions about the relationship of ACF to colorectal cancer cannot be investigated without recourse to careful site studies and must also include a consideration of the histological appearances of the resulting lesions. Our study has shown that ACF are confined to the distal colon where the de novo sequence is invoked to explain the occurrence of poorly differentiated carcinomas. Future studies must relate ACF incidence to both the site and the histotyple of tumor.

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REFERENCES

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