Abstract. The surface morphology of late colonic lesions in F344 rats treated with 1,2-dimethylhydrazine was studied by scanning electron microscopy. At week 31 after carcinogen treatment, the surface epithelial characteristics of different types of lesions observed in the colonic mucosa were compared, namely classic elevated aberrant crypt foci (ACF), flat lesion and gross tumour. Classic elevated ACF were easily observed as structures with enlarged crypts elevated from the background mucosa. When the various ACF were compared, or when the ACF were compared with the background mucosa, no ultrastructural differences, or differences in the density of goblet cells were found. The flat lesion showed an epithelium without goblet cells and crypts with small openings harbouring a large number of loose, undefined, dysplastic epithelial cells. These changes appeared to be linked to the malignant development since they were also characteristic of the examined tumour.

In 1987, Bird et al. (1) introduced a method to view the early development of experimental colon carcinogenesis in rodents. In whole-mount formalin-fixed colon preparations stained with methylene blue, they described how to detect small, protruded lesions by examining the entire mucosal surface under the light microscope. They described aberrant crypt foci (ACF) as putative preneoplastic lesions in the colon of carcinogen-treated rodents. ACF were also observed in the colon of patients with colorectal cancer and in patients with familial adenomatous polyposis (FAP) (2). Since the total number and size (crypt multiplicity) of these small lesions can be scored routinely, ACF have been used as a short-term bioassay to evaluate the role of nutritional components and chemopreventive agents at an early stage of colon carcinogenesis (3). Before the concept of ACF was established, preneoplastic lesions were variously described as: very rare histological changes after extensive serial sectioning of rodent colon (4, 5); minute microadenomas in familial polyposis (6); rare preneoplastic lesions in the background mucosa of colorectal cancer patients (7); foci of early neoplastic changes at the mucosal surface observed by scanning electron microscopy (8-10). Although all these putative preneoplastic lesions seem to be related, it is still not known whether any of them are real precursors of colonic tumours. In azoxymethane (AOM)- or 1,2-dimethylhydrazine (DMH)-treated rodents the number of tumours is minuscule compared with the large number of ACF (2, 11), demonstrating that, in theory, only a very small fraction of the ACF has the potential to progress to the stage of a tumour. Recently, colonic lesions have been described in rodents that seem to be more directly related to tumorigenesis than the classic elevated ACF, i.e., flat dysplastic ACF (11, 12), β-catenin accumulated crypts (13) and mucin-depleted foci (14).

We have previously examined the mucosal surface in F344 rats by scanning electron microscopy two weeks after DMH treatment (15). ACF were seen as structures elevated from the background mucosa. However, there were no ultrastructural variations between the different ACF, or between the ACF and the background mucosa. Scanning electron microscopic examination of the colonic surface of Min mice exposed to azoxymethane revealed two types of ACF (12): classic elevated ACF and flat ACF. Whereas the classic elevated ACF were recognized as distinct and elevated structures with a similar ultrastructure as the surrounding epithelium, the flat ACF were recognized as enlarged and swollen crypts with a relatively flat appearance and loss of goblet cells compared with the background epithelium.

In the present work, scanning electron microscopy was used to study the epithelial surface characteristics of late colonic lesions in F344 rats treated with DMH. At week 31 after carcinogen treatment, the surface morphology of

Abbreviations: DMH, dimethylhydrazine; ACF, aberrant crypt foci.

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different types of lesions observed in the colonic mucosa was compared, namely, classic elevated ACF, flat lesion and gross tumour.

Materials and Methods

Eight male F344/Mol rats from Møllegaard Breeding Center (L.I. Skensved, Denmark), weighing 120 g, were housed in plastic cages in a room with a 12-h light/dark cycle and controlled humidity and temperature and were given free access to a standard diet and water. One hundred and twenty mg/kg body weight of DMH - 2HCl (Aldrich Chemical Company, Germany), freshly dissolved in 0.9% NaCl and 0.18% EDTA at pH 6.5, was injected s.c. twice a week for two weeks.

For scanning electron microscopy, four rats were killed by cervical dislocation 31 weeks after the last DMH injection. The colon was removed, rinsed in ice-cold phosphate-buffered saline (PBS), slit open longitudinally, fixed flat between filter papers in 2.5% glutaraldehyde (GA) in PBS (pH=7.4) for 4 h at room temperature and stained with 0.2 % methylene blue dissolved in the same solution for 20 sec. The whole mount preparations were examined by transillumination under the inverse light microscope. Specimens with colonic lesions were dissected, washed in PBS and dehydrated from a graded series of 70% - 100% ethanol, before critical point drying (CPD, Balzers Union, Liechtenstein) from CO₂. The dried specimens were oriented and mounted on stubs under a stereo microscope, then sputter-coated with platinum. Scanning electron microscopy was carried out using a JSM 840 scanning electron microscope (Jeol, Japan Electron Optical Laboratory, Tokyo, Japan) operated at 15 KeV. Stereo pair electron micrographs were taken with a 100 difference in angle.

For histological examination of lesions in the intermediate stage of colon carcinogenesis, four rats were killed at week 16. Lesions, identified by transillumination and surface examination of formalin-fixed whole-mount preparations under the inverse light microscope, were dissected, embedded in paraffin wax, cut in parallel with the mucosal surface and stained with haematoxylin and eosin (H&E).

Results

At week 31 after DMH treatment, three types of late colonic lesions were observed under the light microscope: classic elevated ACF (Figure 1 A), flat lesions (Figure 1 B) and gross tumours (data not shown). The late classic elevated ACF resembled those observed at week 16 (Figure 1 C); this lesion showed hyperplastic crypts (Figure 1 E). The late flat lesion in Figure 1 B contained more crypts than flat lesions observed at week 16 (Figure 1 D); this lesion showed crypts with severe dysplasia (Figure 1 F).

The same classic elevated ACF observed with the light microscope (Figure 1 A) were examined by scanning electron microscopy (Figure 2 A); these lesions were easily observed as structures with enlarged crypts elevated from the background mucosa. No ultrastructural differences at the cellular level were found when the various ACF were compared, or when the ACF were compared with the background mucosa (Figure 2 E and F). The aberrant crypts had the same density of goblet cells as the crypts of the surrounding background mucosa (Figure 2 C, E and F).

The same flat lesion was examined by both light microscopy (Figure 1 B) and scanning electron microscopy (Figure 2 B). This lesion was not elevated from the surrounding mucosa, as illustrated in the enlargement in Figure 2 D, showing the transition between normal surface epithelium with goblet cells (right side of the image) and the lesion with dysplastic epithelium, which lacked goblet cells (middle and left side of the image). The enlargements of the areas indicated by arrows F and G show the ultrastructure of normal epithelium (Figure 2 F) and dysplastic epithelium of the flat lesion (Figure 2 G). The flat lesion appeared to have smaller crypt openings than the normal surrounding crypts and, around these crypt openings, a large number of loose, undefined epithelial cells were seen (Figure 2 G).

The surface morphology of a large tumour (Figure 3 A) was characterised by disorganised, loosely connected epithelial cells and loss of goblet cells (Figure 3 B and C), as observed in the flat lesion. Small crypts openings were also observed on the surface of the tumour (Figure 3 B). The irregular and loosely connected epithelial cells of the tumour (Figure 3 C) differed significantly from the regular and tightly connected epithelial cells of the surrounding mucosa (Figure 3 D).

Discussion

The surface morphology of three types of late lesions were examined in the colon of DMH-treated rats by scanning electron microscopy, namely, classic elevated ACF, flat lesion and gross tumour.

The observed surface morphology of classic elevated ACF in this late stage of carcinogenesis was, in principle, the same as we had previously reported for the early stages in rats (15), in mice (12) and in hamsters (16): no ultrastructural differences at the cellular level were found when the various ACF were compared, or when the ACF were compared with the background mucosa. This similarity included the frequent and normal presence of goblet cells. Interestingly, these small, elevated lesions had been observed with scanning electron microscopy in DMH- or AOM-induced rat mucosa (8-10), even before the concept of ACF was established. These lesions were described as protuberant gland units, each with a slit-like orifice (8), raised foci centred on crypt orifices (9) and focal protuberances containing numerous abnormal crypts (10). However, the small size and normal surface features of classic elevated ACF, even at a late stage of colon carcinogenesis, indicate that these lesions are not directly related to the tumorigenesis. This is in concordance with our previous conclusions from studies in rats (11) and mice (12).
The flat lesion, examined by scanning electron microscopy at week 31 after DMH, resembled the flat dysplastic ACF we had described in Min mice with the same technique (12, 17) and the dysplastic crypt foci (DCF) Sanchez Negretto et al. (18) had described in DMH-treated rats, also with scanning electron microscopy. The pattern of disorganized and loose epithelial cells and loss of goblet cells was also observed in the surface of the examined tumour, indicating that these changes are linked to the malignant process, and that flat lesions and tumours may be parts of the same continuum. In our previous studies, we hypothesized that flat ACF and tumours may represent the same type of dysplastic lesions at different stages of crypt multiplication, because there was a uniform picture of severe dysplasia including loss of goblet cells, as well as aberrant Wnt activation in the small and large lesions (11, 12).

In conclusion, classic elevated ACF displayed a uniform and normal appearing ultrastructural surface, supporting the hypothesis that these lesions are not directly associated with colon carcinogenesis. In contrast, the flat lesion examined,
Figure 2. Scanning electron microscopy of the colonic mucosa of F344 rats, after DMH treatment. A) Classic elevated ACF, recognised as distinct structures with epithelial cells of similar ultrastructure as the surrounding mucosa (see also Figure 1A). This is illustrated in C) by enlarging the ACF indicated by the arrow C. Normal epithelium with goblet cells are illustrated in E), by enlarging the area indicated by the arrow E. B) A flat lesion recognised as an area with small crypt openings and loss of goblet cells (see also Figure 1B). D) Illustrates the transition between normal epithelium with goblet cells (left side of image) and the flat lesion demonstrating dysplastic epithelium with loss of goblet cells (middle and right side of the image). The areas indicated by the arrows F and G are enlarged to show the normal epithelium F) in contrast to the dysplastic epithelium of the flat lesion G). Magnifications: 50x, 200x and 1125x.
which resembled the previously described flat ACF, showed ultrastructural changes that were also characteristic of the examined tumour, supporting the hypothesis that flat ACF are directly linked to malignant development. However, further scanning electron microscopic studies are needed to describe the ultrastructural changes from small to large lesions.

References


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