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Colonic mucosal changes in nude mice associated with orthotopic xenografts of human colon cancer cells

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Summary. We used the nude mouse tumour xenograft model to study the pathogenesis of mucosa alterations in the large bowel surrounding a carcinoma. In mouse colonic mucosa overlying HT-29 colonic carcinoma xenografts in the caecum, the crypts were elongated in comparison with those in distant mucosa and also frequently showed a shift towards sialomucin production. These features, which are comparable with socalled transitional mucosa (TM) in man, were absent in control animals inoculated with Indian Ink instead of HT-29 cells. Although the localization of the proliferative cell compartment in mouse colonic mucosa overlying HT-29 xenografts appeared to be confined to the lower half of the crypt as in normal mucosa, the relative length of the DNA synthesizing cell compartment along the crypts was slightly elongated. These data strongly suggest that TM should be regarded as a secondary phenomenon rather than a premalignant change in large intestinal epithelium and that higher proliferative activity of epithelial cells contributes little to the elongation of crypts in TM.

Key words: Transitional mucosa – Colon carcinoma – Nude mice – Xenografts

Introduction

Filipe (1969) first described morphological and histochemical changes in large intestinal mucosa adjacent to adenocarcinomas for which she introduced the term "transitional mucosa" (TM). Morphologically, TM shows elongated and occasionally branched crypts lined by abundant goblet cells. Histochemically, goblet cells of TM frequently contain sialomucins, whereas mucin producing cells of normal colonic mucosa demonstrate sulphomucin synthesis predominantly. The existence of TM has been amply confirmed in the literature.

What causes TM in large intestinal mucosa and whether or not its occurrence is specifically associated with neoplasia, however, has been subject to sustained controversy. Filipe and co-workers (Penelope and Filipe 1976; Greaves et al. 1980; Lapertosa et al. 1984) on the basis of the biochemical characteristics of mucin produced in TM have postulated that TM represents a premalignant alteration of large bowel mucosa. These investigators identified small foci of TM in the mucosa of adenoma patients and also in the unaffected mucosa of familial polyposis patients which could be regarded as an argument in support of this opinion. Others (Lev et al. 1985) have confirmed some of the above findings and observed TM in other conditions associated with a risk of large bowel cancer, such as ureterosigmoidostomy (Marcheggiano et al. 1983). Isaacson and Attwood (1979) were the first to dispute these views and subsequently were followed by many other investigators (Listinsky and Riddell 1981; Franzin et al. 1983; Lanza et al. 1985). The criticism of these authors was mainly based on the observation that TM changes occur also in the mucosa adjacent to mesenchymal tumors, metastatic tumor, diverticula and the solitary rectal ulcer syndrome.

Animal models could be of value in elucidating the pathogenesis and biological significance of TM, but these have hardly been employed to this effect. Filipe (1975) observed predominant sialomucin production and characteristic alterations in cytonuclear morphology in the mucosa of dimethylhydrazine (DMH) treated rats prior to development of overt carcinoma. We have investigated the morphology, mucin characteristics and proliferative compartment of mouse colonic mucosa overlying intramucosal xenografts of HT-29 colon carcinoma cells.

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Materials and methods

Athymic CD-1 male nude mice, 4–8 weeks old, were obtained from Charles River Wiga (West Germany). They were maintained in a laminar air-flow cabinet under specific pathogen free conditions.

For the purpose of this study the HT-29 cell line derived from a colon carcinoma and a primary human fibroblast culture were used. The cell lines were maintained in Dulbecco's Modified Eagle's Medium supplemented with 10% fetal calf serum. For injection tumour cells and fibroblasts were dispersed with 0.1% trypsin in 0.02% EDTA, washed with sterile PBS, and single cells were prepared in a concentration of 2×10^7/ml PBS. Cell viability was determined by the trypan blue exclusion method.

After ether anesthesia, a small median abdominal incision was made. The caecum was easily pulled out from the abdominal cavity without any major bleeding. Tumour cells, fibroblasts (1×10^6 in 0.05 ml PBS) or 50 µl. Indian Ink were injected into the caecal wall at the insertion of the mesocolon using a 30G needle (Becton & Dickinson). After the inoculation the abdomen was closed in two layers.

The mice were killed 50 days after the inoculation of tumor cells, fibroblasts or Indian Ink. The caecum was removed, fixed in Periodic acid-Lysine-Paraformaldehyde and embedded in paraplast. 4 µm sections were stained with H&E and with the High Iron Diamine Alcian Blue method at pH 2.5 (modified method of Spicer (1965)) for identification of sialomucins and sulphomucins.

The length of the crypts was measured morphometrically using a MOP videoplan (Kontron, West Germany) electronic image digitizer. The mean crypt length was determined by measuring 5 crypts in the mucosa overlying the xenograft. Repeated measurement of the same specimen confirmed the reproducibility of the measurements. For measurement areas were carefully selected where the section was cut perpendicular to the mucosal surface. Normal crypt length was determined in mucosa distant from the xenograft.

To determine the proliferation characteristics of the mucosa, the mice were injected with Bromodeoxyuridine (BrdUrd, Serva, Heidelberg), 50 mg/kg body weight by intraperitoneal administration 5 h before sacrificing the animal. BrdUrd incorporating cells were visualized by means of an immunoperoxidase method, using anti-BrdUrd antibody as described previously (Schutte et al. 1987). The relative length of the DNA synthesizing cell compartment along the crypt was calculated by the following equation:

$$\frac{\text{Distance crypt bottom level highest labeled cell}}{\text{total crypt length}} \times 100\%$$

Results

From the 5 mice inoculated with HT-29 colonic carcinoma cells, 4 were used for this study. One mouse had to be excluded from the experiment because of death from ileus 30 days after injection of the HT-29 cells. Two mice were injected with Indian Ink as a control. On sacrifice 50 days after HT-29 injection all animals showed a tumour nodule in the caecal wall at the site of inoculation. Microscopically the tumours appeared to be poorly differentiated. They invaded into the overlying colonic mucosa and demonstrated a predominance of sialomucin production. Comparison of the overlying mucosa with the mucosa remote from the xenograft revealed the presence of significantly longer crypts in the former ($p < 0.01$) (Table 1; Fig. 1 and 2). In 3 out of 4 mice the mucosa overlying the xenograft displayed sialomucin production in some goblet cells against a background of predominance of sulphomucin production (Fig. 3) whereas sialomucin was not observed in the distant mucosa (Fig. 4). Table 2 shows a comparison of the relative length of the proliferative cell compartment along the crypts of the overlying and the remote mouse mucosa. Although not statistically significant, the overlying mucosa demonstrated a trend towards a slightly longer proliferating zone than the distant mucosa (Fig. 5 and 6). The two animals inoculated with human fibroblasts and the two injected with Indian Ink, in contrast, did not demonstrate morphological alterations of the mucosa overlying the inoculation site as compared to the remote mucosa. A shift from sulpho – to dialomucin production was not observed.

Table 1. Characteristics of mouse mucosa overlying and remote from the xenograft

Mouse	Mucin production		Length of crypt[c] (µm)	
	Distant mucosa	Overlying mucosa	Distant mucosa	Overlying mucosa
1	sulpho[a]	sialo[b]/sulpho	162.9 ± 14.2	253.9 ± 73.5
2	sulpho	sulpho	197.0 ± 5.3	222.2 ± 9.9
3	sulpho	sialo/sulpho	182.9 ± 15.0	280.4 ± 27.0
4	sulpho	sialo/sulpho	189.9 ± 10.8	370.3 ± 63.5
			$p < 0.01$	

[a] sulpho: sulphomucin
[b] sialo: sialomucin
[c] The crypt length was measured morphometrically. Each value shows the mean ± SD of 5 crypts' length

Table 2. The relative length of the BrdUrd labeled cell compartment along the crypts

Mouse	Distant mucosa	Overlying mucosa
1	50.9 ± 5.3 (%)	65.7 ± 9.5 (%)
2	39.8 ± 6.2	40.3 ± 9.4
3	45.2 ± 2.9	52.3 ± 11.9
4	45.8 ± 5.8	51.3 ± 5.0

Each value demonstrates the mean ± SD of 5 crypts

Discussion

The data presented in this study show that the morphological features characteristic of TM develop in the mucosa overlying a human colon tumour transplant 50 days after implantation, whereas these features were not present in the distant mucosa and the overlying mucosa of control animals inoculated with human fibroblasts or Indian Ink. Alterations in the type of mucin produced, typical of TM, were shown to occur in the mucosa overlying the HT-29 xenografts in 3 out of 4 mice and not in control animals. From these findings we feel that TM changes should be considered as secondary to the existence of a carcinoma. In addition, our data suggest that the morphological and histochemical features of

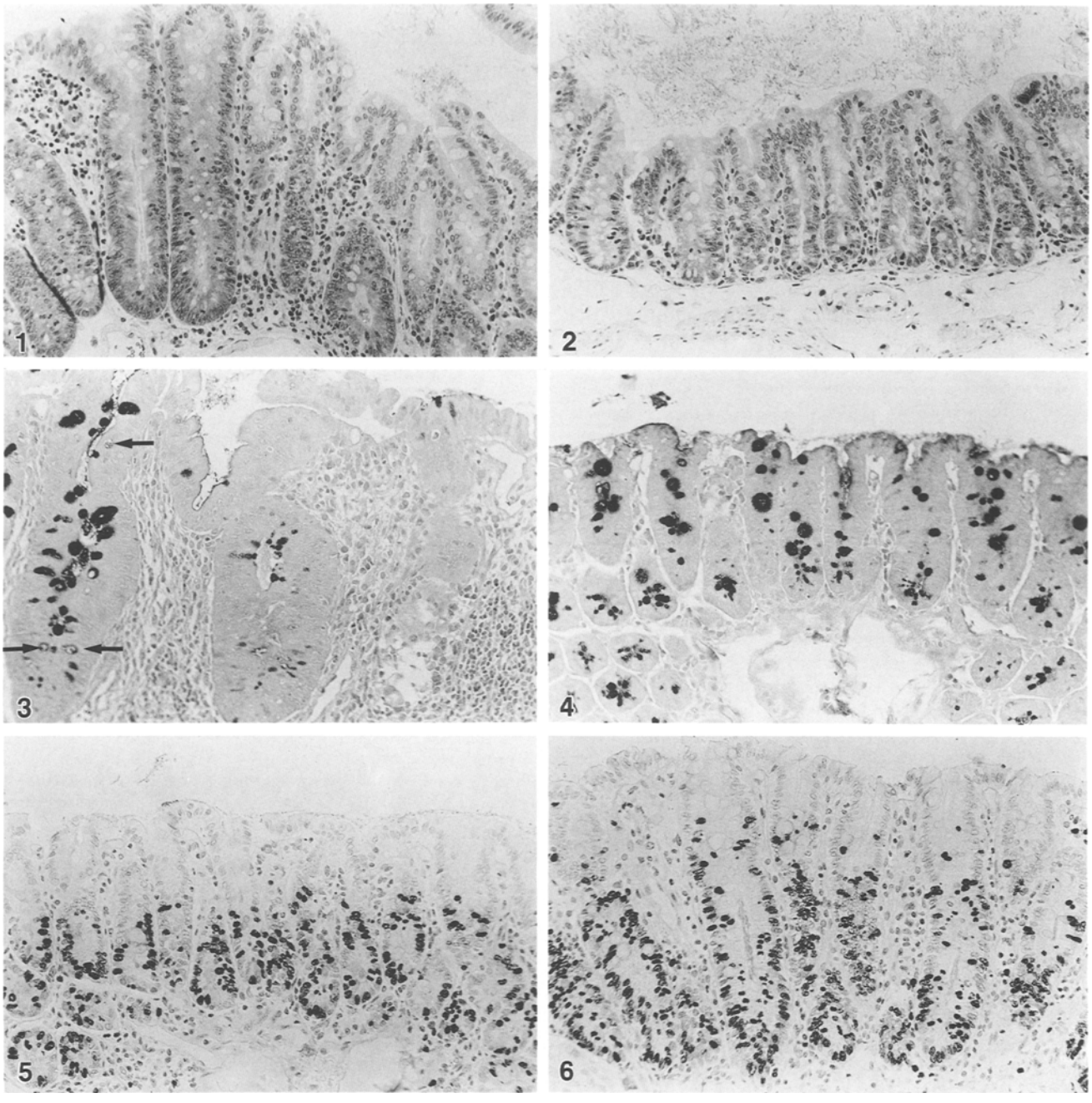

Fig. 1. Nude mouse caecal mucosa overlying a HT-29 xenograft (H/E, ×150)

Fig. 2. Nude mouse caecal mucosa distant from a HT-29 xenograft at the opposite site in the caecal wall (H/E, ×150)

Fig. 3. Nude mouse caecal mucosa overlying a HT-29 xenograft. Some sialomucin producing goblet cells can be seen (arrows) against a background of predominant sulphomucin production (HID-AB stain, ×250)

Fig. 4. Nude mouse caecal mucosa distant from a HT-29 xenograft demonstrating exclusive sulphomucin production in goblet cells (HID-AB stain, ×250)

Fig. 5. Proliferative cell compartment of nude mouse caecal mucosa overlying a HT-29 xenograft (immunocytochemistry after Brd Urd labeling, ×150)

Fig. 6. Proliferative cell compartment of nude mouse caecal mucosa distant from a HT-29 xenograft (immunocytochemistry after Brd Urd labeling, ×150)

TM do not always occur together, the morphological features of TM occasionally preceding the changes in mucin production.

Lipkin (1974) demonstrated DNA synthesizing cells along the full length of the crypt epithelium in patients with precancerous familial polyposis and in the DMH mouse model using ^{3}H-thymidine incorporation followed by autoradiography, whereas proliferating cells in normal mucosa were confined to the lower crypt portion. Our cell kinetic observations demonstrate that the localization of BrdUrd labelled cells in both mucosa with and without TM features was confined to the lower half of the crypt. These data provide another indication that TM is not of a preneoplastic nature. We observed a slightly, but not significantly, longer proliferative cell zone in the mouse mucosa overlying the HT-29 xenograft as compared to the mucosa remote from the xenograft, which suggests that enhanced proliferation may be involved in the pathogenesis of TM. The main factor, however, influencing the morphological alterations in TM, according to our data, appears to be delayed maturation and detachment of cells from the mucosal surface.

What exactly causes the development of TM is still unknown. It is attractive to speculate on the possible role of transforming growth factors (TGF-s), the production of which has been demonstrated in various cancer cells, including colorectal carcinoma cells (Coffey et al. 1986). Recent studies have shown that TGFs can confer the transformed phenotype onto non-neoplastic cells by influencing proliferative behaviour and differentiation, in part through modification of the number of plasma membrane receptors for epidermal growth factor (EGF) (Marks and Brattain 1984; Assoian et al. 1984). Evidently, further experiments are necessary to elucidate the pathogenesis of TM alterations in colorectal cancer.

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