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Cell proliferation and differentiation in intramucosal and advanced signet ring cell carcinomas of the human stomach

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Summary. In order to study the progression of signet ring cell carcinomas in the human stomach, we compared cell proliferation and differentiation between small and large intramucosal cancers, and between intramucosal and advanced cancers. Fine-structurally, signet ring cells were differentiated to 3 cell types: a foveolar, a glandular and an intestinal type. In the mucosa, the foveolar-type cells and glandular-type cells were distributed at the superficial and the deep zone, respectively. In the small mucosal cancers, intestinal-type cells were rare and a layered structure was often seen. In this structure, the mode of cell production resembled that in the normal gastric mucosa; the foveolar-type signet ring cells in the superficial layer were not proliferative and the proliferating cells were small cells in the middle layer and a few glandular-type cells in the deep layer. In the large mucosal and advanced cancers, intestinal-type cells and proliferating small round cells were often distributed throughout the depth of the mucosa, and signet ring cells of the foveolar type were also proliferative. These findings indicated that large part of the signet ring cell carcinomas initially form the layered structure and that it becomes indistinct while intestinal-type cells appear as the tumour grows. However, we found several small advanced cancers, lacking both the layered structure and the intestinal-type cells. These cancers appear to start without the layered structure and progress very rapidly.

Key words: Stomach – Signet ring cell carcinoma – Tritiated thymidine autoradiography – Cell differentiation – Cancer progression

Introduction

Signet ring cell carcinoma is classified as diffuse type by Lauren (1965), infiltrative type by Ming (1977) or undifferentiated carcinoma by Sugano et al. (1984). Cytologically, this carcinoma is well differentiated, producing mucous cells. It was recently described that, in early signet ring cell carcinomas a layered structure is often seen (Hattori et al. 1984; Katsuyama et al. 1985), comprising 3 layers; a superficial and a deep layer composed of signet ring cells and a middle layer composed of small round cells. Using experimental signet ring cell carcinomas, we have demonstrated that the small cells in the middle layer proliferate to produce the signet ring cells of the superficial and the deep layer (Sugihara et al. 1985). This mode of cell production resembles the cell renewal pattern of the normal gastric mucosa. When cancers grow to form the layered structure, they appear to spread at the gland neck level of the mucosa and seldom progress to an advanced cancer.

In the human stomach, various patterns of tumour growth such as the superficially spreading type (Stout 1942; Myhre 1953; Mason 1965) and a deeply infiltrative or penetrating type (Kodama et al. 1983) have been described. In signet ring cell carcinomas, these different growth patterns may be related to the occurrence of a layered structure; it can be supposed that widely-spreading mucosal cancers might well retain the typical layered structure. In order to study this point, we compared an incidence of the layered structure and cell types between the small mucosal cancers and superficially spreading or advanced cancers. To identify the layered structure both histologically and from the cytokinetic point of view, we labeled surgically-obtained cancer tissues in vitro with ^{3}H-thymidine, and carried out autoradiographic studies. To determine cell types, electron microscopic and mucin-histochemical studies were also performed.

Materials and methods

We have examined 68 resected stomachs with signet ring cell carcinoma, excluding mucinous or less differentiated adenocarcinoma with signet ring cells. The specimens were taken in pre-

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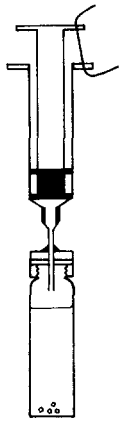


Fig. 1. A simple hyperbaric chamber made of a disposable syringe and a screw vial

fectures of Kyoto and Fukui between 1981 and 1986. For determination of extent of mucosal spread and depth of invasion in each case, the entire cancer lesion was step-sectioned at about 7-mm width and conventional histological studies were carried out. In this study, we used 30 lesions (Table 1): 21 intramucosal cancers with or without minute submucosal invasions (of less than 1 mm in diameter) and 9 advanced cancers with the mucosal lesion smaller than 4 cm in diameter, in which a diffusely infiltrative (scirrhous) carcinoma was included (case No. 23). Cases of early cancers with larger submucosal invasions and advanced cancers of larger mucosal lesions were not studied in the present paper.

Within 60 min of resection, several pieces of mucosal cancer tissues of about 0.5 cm × 0.5 cm in size were sampled and placed immediately in ice-cold Eagle MEM medium (Nakarai Chemical, Kyoto, Japan). They were cut into small tissue blocks

Table 1. Proliferative activity and type of signet ring cells as well as intramucosal distribution of small cancer cells. Proliferative signet ring cells are marked with asterisks. Small cancer cells are distributed in the middle layer (*M*) or diffusely throughout the depth of the mucosa (*D*). Co-existence of the both patterns is expressed as *D/M* and *M/D*, which show a predominant pattern is D and M, respectively. In the layered structure, the small cells are confined to the middle layer and signet ring cells of the foveolar-type are not proliferative. Numerals in parenthesis show percentages of alcian-blue-positive (intestinal-type) cells in the mucosal cancer cells.

	Case no.	Age	Sex	Mucosal spread (cm)	Depth of invasion	Signet ring cells			Distribution of small cancer cells
						Foveolar type	Glandular type	Intestinal type	
Small mucosal type	1	48	M	1.3 × 0.7	m	+	+	−(< 5)	M
	2	39	F	1.7 × 1.0	m	+	+	+(< 5)	M
	3	49	F	2.0 × 1.2	m	+	+	−(< 5)	M/D
	4	65	M	2.0 × 1.5	m	+	+*	−(5–10)	M
	5	40	M	2.0 × 1.5	m	+	+	−(< 5)	M
	6	37	F	2.0 × 1.7	m	+*	+	+*(10–25)	M/D
	7	55	F	3.0 × 1.5	m	+	−	−(< 5)	D/M
	8	53	F	3.0 × 2.5	m	+	+	−(< 5)	M/D
	9	38	F	3.0 × 2.5	m	+	−	−(< 5)	M/D
	10	52	M	3.0 × 3.0	m	+*	+	−(< 5)	M/D
	11	41	M	3.2 × 1.3	m	+	+*	−(< 5)	M/D
	12	46	F	3.5 × 1.8	sm (min)	+	+	−(< 5)	M/D
	13	47	F	3.5 × 3.5	m	+	+	+(10–25)	D/M
Superficially spreading type	14	49	F	4.0 × 2.5	m	+*	−	+(25–50)	D/M
	15	63	F	5.0 × 3.0	m	+	+*	+(< 5)	M/D
	16	45	F	5.0 × 3.0	m	+	+	+(< 5)	D/M
	17	32	F	5.0 × 4.0	sm (min)	+*	+	+(< 5)	M/D
	18	42	F	5.0 × 4.5	sm (min)	+*	+	+(10–25)	M/D
	19	47	M	8.0 × 6.5	m	+*	+	+(5–10)	D/M
	20	66	F	8.0 × 7.5	m	+*	+	+(5–10)	D/M
	21	31	F	9.0 × 7.5	m	+*	+*	+*(25–50)	D/M
Deeply infiltrating type	22	69	M	0.8 × 0.8	pm	+*	+	+(25–50)	D/M
	23	42	F	1.0 × 1.0	ss (diffuse)	+*	+	−(< 5)	D
	24	45	M	1.6 × 1.5	ss	+*	+	−(< 5)	D/M
	25	56	F	1.9 × 1.5	pm	+*	−	−(< 5)	D
	26	48	F	2.0 × 1.2	pm	+*	+*	−(< 5)	M/D
	27	41	M	2.5 × 2.0	ss	+*	+*	−(10–25)	M/D
	28	42	F	3.0 × 2.0	ss	+*	+*	+(25–50)	D/M
	29	29	F	3.0 × 2.5	ss	+*	−	+(10–25)	D/M
	30	39	F	3.7 × 3.5	ss	+*	+	+(5–10)	M/D

m = mucosa; sm = submucosa; pm = proper muscle layer; ss = subserosa

of not larger than 1 mm^3, and were transferred to an incubation chamber, which was supplemented with a screw vial and a disposable syringe (Fig. 1). It is known that oxygenation of tissues is necessary for DNA synthesizing cells to incorporate ^{3}H-thymidine in vitro (Steel and Bensted 1965). The optimal oxygen pressure was described to be 3–4 atm (Fabricant et al. 1969). To obtain this condition, we filled the 5-ml vial with 4 ml of incubation medium containing 10 μCi/ml ^{3}H-thymidine (sp. act. 5 Ci/mM; Amersham International, Amersham, UK), and

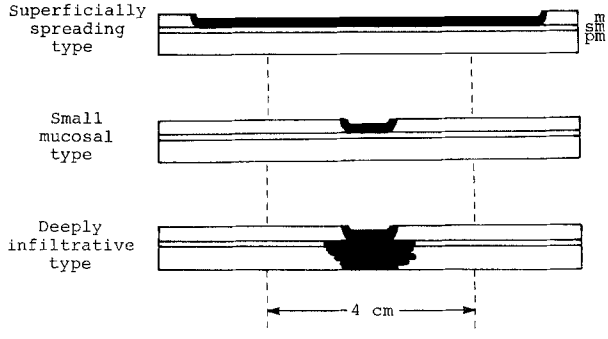


Fig. 2. Growth patterns of signet ring cell carcinomas. m: mucosa, sm: submucosa, pm: proper muscle layer

then plunged 3 ml of pure oxygen into the vial. The syringe was clamped with a short wire during the incubation. After incubating for 20 min, we unfastened the wire and measured the recovery of air volume to determine whether leakage had occurred.

The tissues were fixed in 2% glutar-/2% paraform aldehyde in 0.05 M cacodylate buffer (pH 7.4) for 6 h at 4° C. After overnight washing in the same buffer, they were postfixed in 1% osmium tetraoxide for 1 h at room temperature, dehydrated in a graded series of ethanol and embedded in epoxy resin (Quetol 812; Nissin EM, Tokyo, Japan). Among many resin blocks of tissue, those providing the sections cut perpendicularly to the mucosal surface were selected. After removal of resin from the semithin section of the selected blocks in NaOH-saturated absolute ethanol for 5 min (Imai et al. 1968), the semithin sections were dipped in nuclear emulsion (Kodak NT-B2), exposed for 4 weeks at 4° C and developed in FD-111. The autoradiographs were counterstained with toluidine blue at room temperature. We further selected adequately-labeled tissues from which we made serial ultrathin and semithin sections. After making autoradiographs from these semithin sections and staining the ultrathin sections with uranyl acetate and lead citrate, we observed the fine structure of the cancer cells by an electron microscope (Hitachi H-600), checking their proliferative activity in the corresponding autoradiographs.

Electron micrographs of 20 to 60 labeled cancer cells were

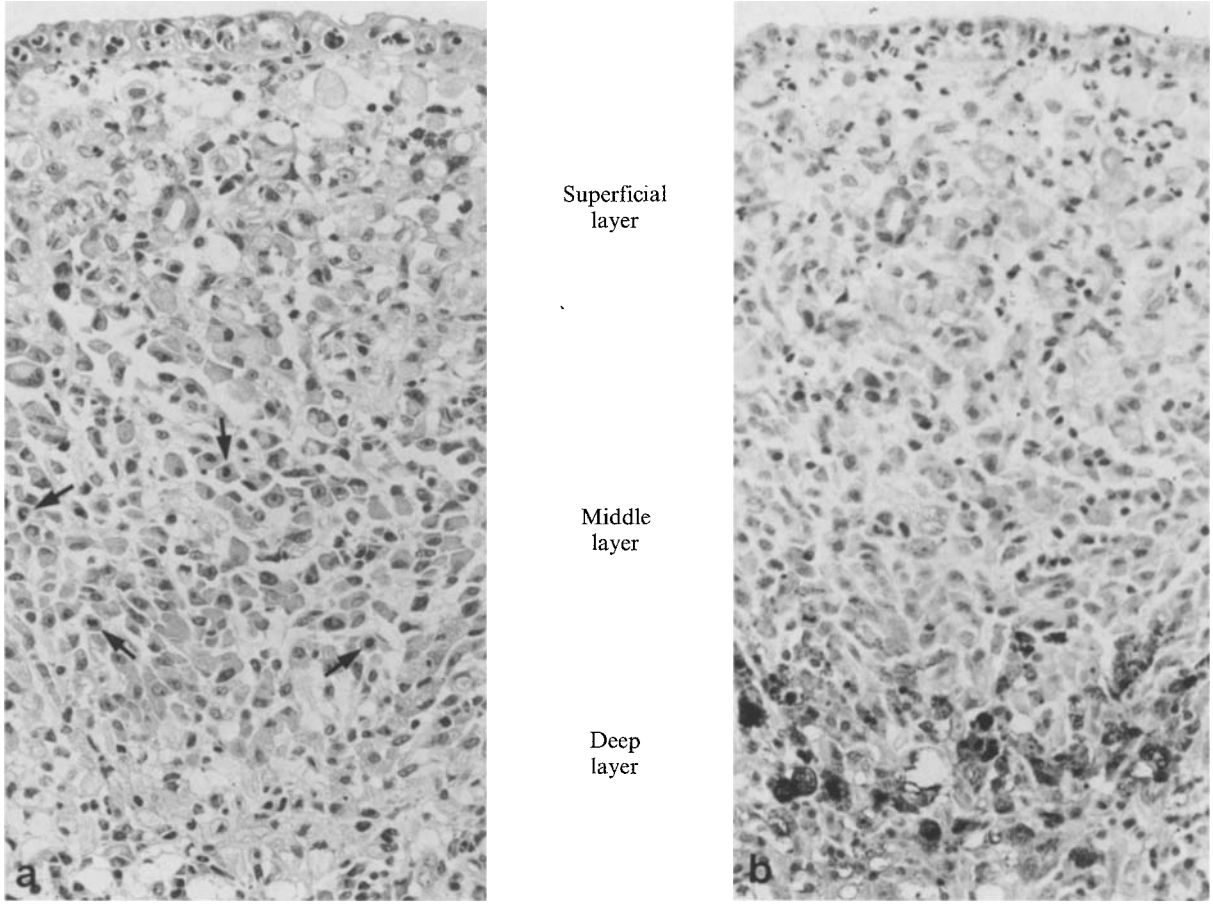


Fig. 3a, b. The layered structure in a small intramucosal signet ring cell carcinoma (case No. 3). **a** The layered structure is composed of a superficial and a deep layer of signet ring cells and a middle layer of small round cells. Mitotic cancer cells (*arrows*) are confined to the middle layer. HE-stain ×260. **b** Signet ring cells in the deep layer are positively stained with the class III Con A method. ×260

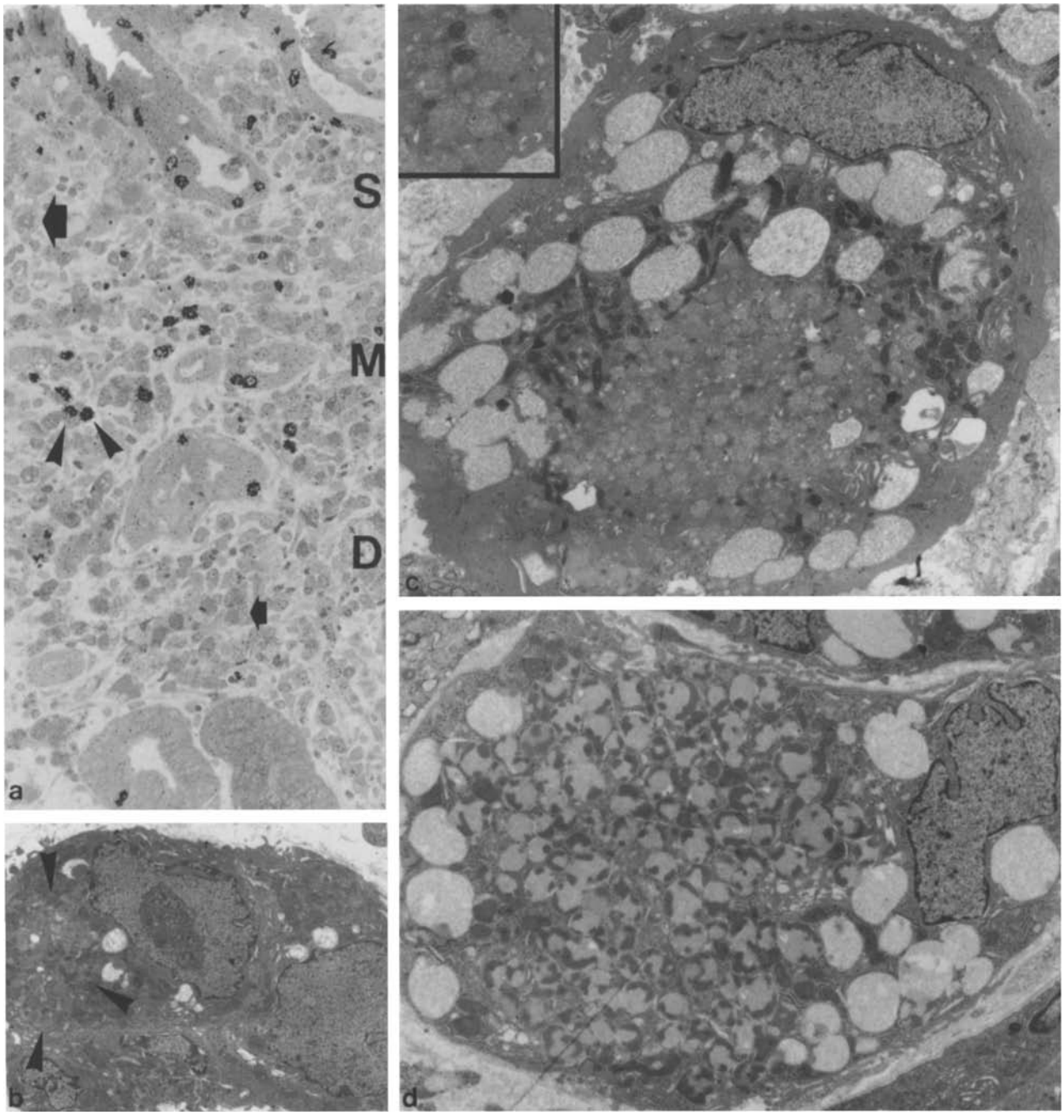


Fig. 4a. An autoradiograph of the layered structure (case No. 11). ^{3}H-thymidine incorporating cancer cells are small in size and are distributed in the middle (*M*) layer of the mucosa. Signet ring cells in the superficial (*S*) and the deep (*D*) layer are not labeled. Labels in the superficial stroma are on lymphocytes. Remaining pits are hyperproliferative. Toluidine blue. × 250. **b** Labeled cancer cells in the middle layer (marked with arrow heads in **a**), one of which is immature glandular-type cancer cell with dense mucous granules (*arrow heads*). Total cross-sectional area of mucin is 4.5 μm^2. × 3200. **c** A foveolar-type signet ring cell in the superficial layer (marked with large arrow in **a**) with coarsely stippled mucous granules and pale degenerative granules. The inset is higher magnification of mucous granules. × 5600. **d** A glandular-type signet ring cell in the deep layer (marked with small arrow in **a**), having mucous granules with cores and degenerative granules. × 5600

taken randomly from each lesion for the determination of the mucin area. After mucous granules were painted red on the micrographs, the total cross-sectional area of the granules was measured by a color image analyzer after a method of Fujita (1983).

Other series of serial ultrathin and semithin sections were made for investigation of histochemical properties of various mucous granules. Positive staining with a concanavalin A-horse radish peroxidase (Con A-HRP) method after an oxidization with periodate, followed by a reduction with sodium borohydrate (class III Con A method, Katsuyama and Spicer 1978) is regarded as a marker for glandular-type mucin of the stomach. After removing the resin, semithin sections were stained with a class III Con A method and alcian blue (pH 2.5), and the histochemical and fine structural findings obtained from the same cells were compared. Although osmification of the tissue inevitably masks sugar residues reactive to the class III Con A staining, reactivity was found to be partially recovered by a prolonged oxidation with periodate for up to 2 h.

To check whether or not the small pieces of tissue for fine structural examinations represent the entire lesion, we stained paraffin sections with alcian blue (pH 2.5) and the class III Con A method, and studied the composition of cell types in the entire lesion.

Results

Twenty-one patients out of 30 (70%) were female with ages from 29 to 69 years (Table 1). The mucosal cancers and the mucosal lesions of the advanced cancers examined ranged in size from 1.3 cm to 9.0 cm and from 0.8 cm to 3.7 cm in maximal diameter, respectively. Following Kodama et al. (1983), mucosal cancers were divided into the superficially spreading type (with a diameter of over 4 cm) and the small mucosal type (with the maximal diameter smaller than 4 cm) as shown in Fig. 2.

In paraffin sections, a layered structure was often seen in the peripheral part of the mucosal lesion. The typical layered structure was composed of 3 layers; a middle layer composed of small round cells and a superficial and a deep layer of signet ring cells (Fig. 3a). Mitotic cancer cells were confined to the middle layer (arrows in Fig. 3a). The signet ring cells in the deep layer were stained with the class III Con A method (Fig. 3b). However, the deep layer was lacked in 5 cases, irrespective of tumour size.

Autoradiographically, we defined the layered structure as having a middle layer of small cells labeled with ^{3}H-thymidine and a superficial layer of non-labeled signet ring cells (Fig. 4a) irrespective of the presence of the deep layer. When the deep layer was present, there were occasionally a few labeled signet ring cells in this layer. Label with ^{3}H-thymidine was observed in 2–10% of the small cells in the middle layer, which often had a small number of mucous granules, not more than 10 μm^2 in total cross-sectional area (Fig. 4b).

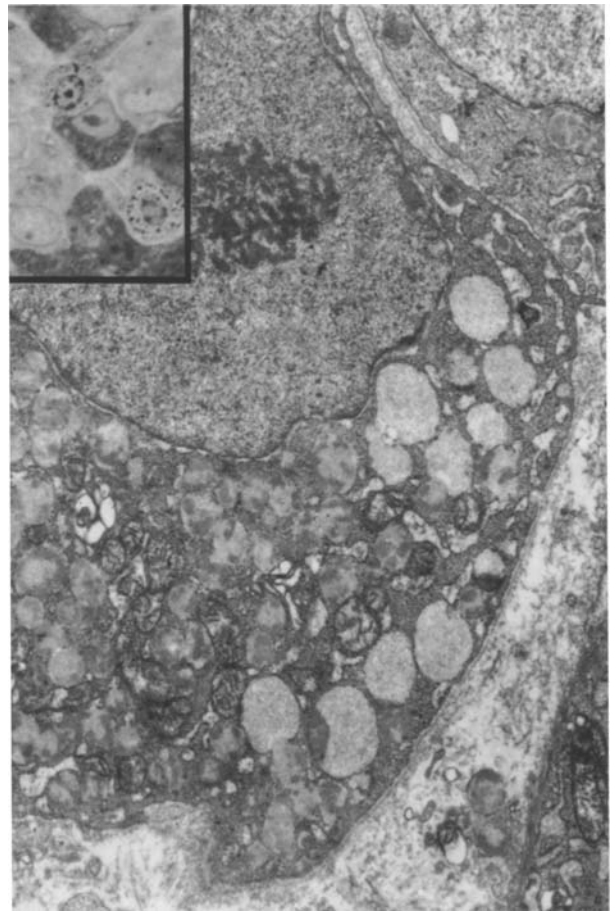


Fig. 5. A signet ring cell in the deep layer, positively stained with class III Con A method in the adjacent semithin section (*inset*), has mucous granules with cores. ×9600

There were different types of signet ring cells either filled with mucous granules or occupied by intracytoplasmic microcysts. In the present study, the signet ring cells of the former type were tentatively defined in electron micrographs as neoplastic cells having mucous granules more than 20 μm^2 in a total cross-sectional area (full blown signet ring cells had mucous granules up to about 30–50 μm^2 in total cross-sectional area as shown in Fig. 4c, d).

Most of the signet ring cells in the superficial part of the layered structure had oval mucous granules with a coarse stippled appearance (Figs. 4c and 7c). Most of the cancer cells with these granules were not stained or very weakly stained with alcian blue in the adjacent semithin sections. As these mucous granules resembled those of the surface mucous cells in gastric foveolae, described by Lillibridge (1964) and Rubin et al. (1968), we considered them as a foveolar type. A small number of these granules of a smaller size

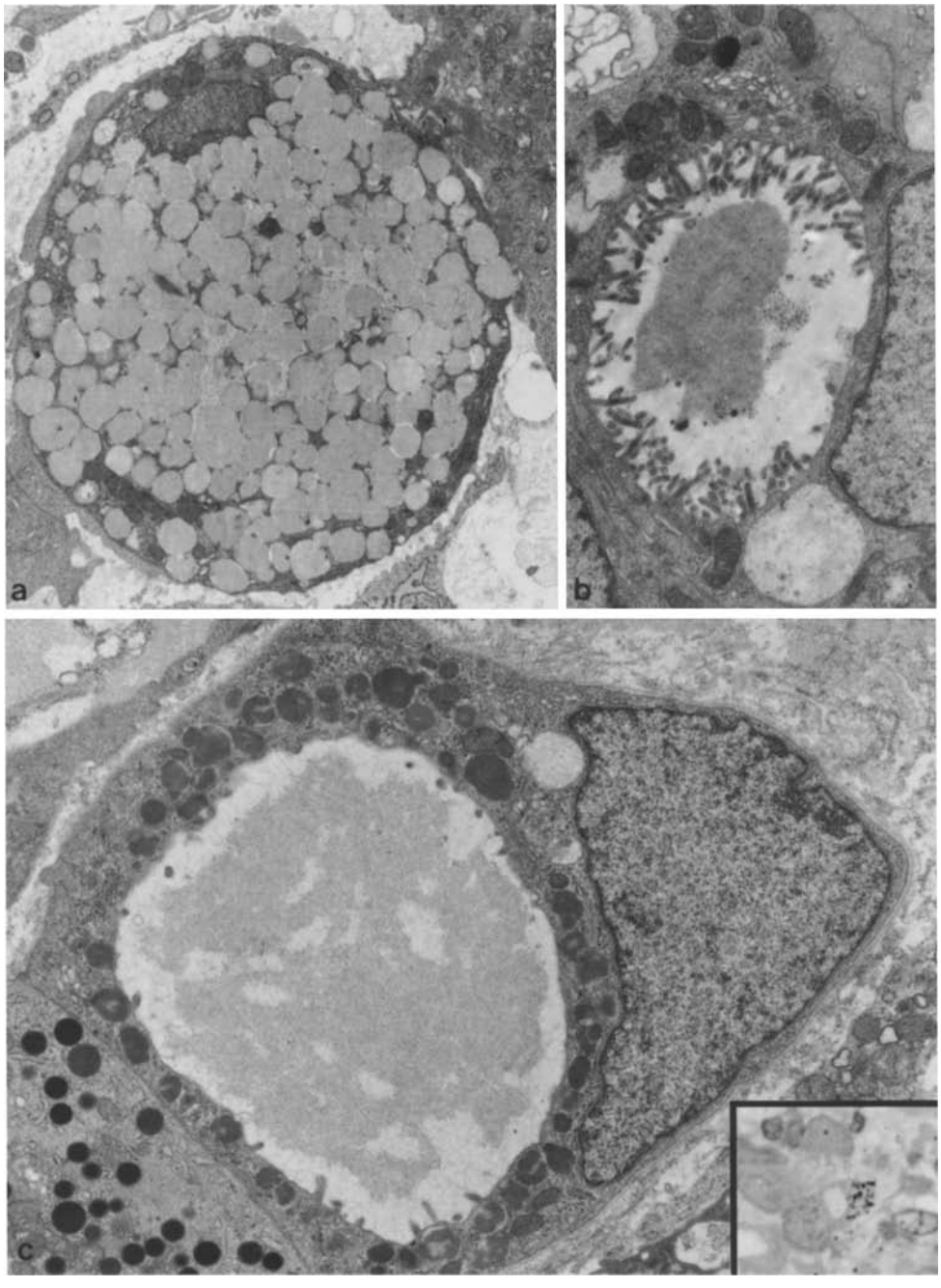


Fig. 6a, b. Intestinal-type cancer cells. **a** A goblet-cell type signet ring cell with pale, homogeneous mucous granules (case No. 13). × 5600. **b** A cell with a microcyst, densely lined by microvilli with prominent core filaments. × 8000. **c** A labeled glandular-type signet ring cell with a microcyst lined sparsely by microvilli and dense mucous granules (case No. 21). × 8000

were often observed in the small round cells in the middle layer.

Cancer cells in the deep part of the layered structure had round mucous granules with a number of irregularly-shaped, eccentric, dense cores (Fig. 4d). Cancer cells having these granules showed class III reactivity to Con A, a marker of glandular (pyloric/mucous-neck cell) type mucin, in the adjacent semithin section (Fig. 5). Therefore, we regarded the cells with these granules as glandular in type. In the small round cells in the middle layer, mucous granules of this type were rarely seen, but there were a few labeled cells in the middle layer having small, dense mucous granules (Figs. 4a and 6c). There were transitional forms from the dense granule to the granule with cores, and the former may be the immature form of the latter.

The third type of the mucous granule was round in shape and filled with pale, homogeneous matrix (Fig. 6a). The signet ring cells with these granules were stained intensely with alcian blue in the adjacent semithin section. These cells resembled goblet cells as described by Trier (1963) and Goldman and Ming (1968), and we regarded them as an intestinal type.

The cancer cells with microcysts could also be classified into the 3 types described above and were found in all the 3 layers of the layered structure. There were microcysts densely lined by microvilli with prominent core filaments (Fig. 6b). The microcysts of this type corresponded to those described by Nevalainen and Jarvi (1976) and Tokumitsu et al. (1978) as an intestinal type. The cancer cells with the mucous granules of the foveolar or the glandular type occasionally had the microcysts with sparse microvilli (Fig. 6c).

Signet ring cells in the uppermost and lowermost zone of the layered structure often had a number of larger granules in addition to those described above (Fig. 4c, d). These granules had a tendency to fuse with each other, contained sparse, flocculent materials in electron-transparent matrix, and sometimes resembled empty vacuoles. The cancer cells filled with these vacuolar granules, which have been described as type C signet ring cells by Yamashiro et al. (1977), were not labeled with ^{3}H-thymidine, whereas the proliferative signet ring cells had a few of these granules (Fig. 7c). We regarded them as a degenerated form of various mucous granules.

In 12 out of the 13 cancers of the small mucosal type, the autoradiographically-defined layered structures were found (Fig. 4a). The signet ring cells in the superficial and the deep layer were foveolar and glandular in type, respectively (Fig. 4d). In several parts of 9 small mucosal cancers (Nos. 3 and 6–13) small cancer cells were not confined to the middle layer, but they were distributed throughout the mucosa. The intestinal-type cells were infrequently seen except for the case No. 13, in which they were distributed randomly in the mucosa. Less then 5% of the cancer cells were stained intensely with alcian blue, except for the cases, No. 4, No. 6 and No. 13. The occurrence of the intestinal-type cells in the mucosal lesion was not related to the degree of intestinal metaplasia in the surrounding mucosa; several cancers lacking the intestinal type cells were found in heavily intestinalized mucosa, and as in the case No. 13, the tumour with many intestinal-type cells was located in fundic mucosa without intestinal metaplasia.

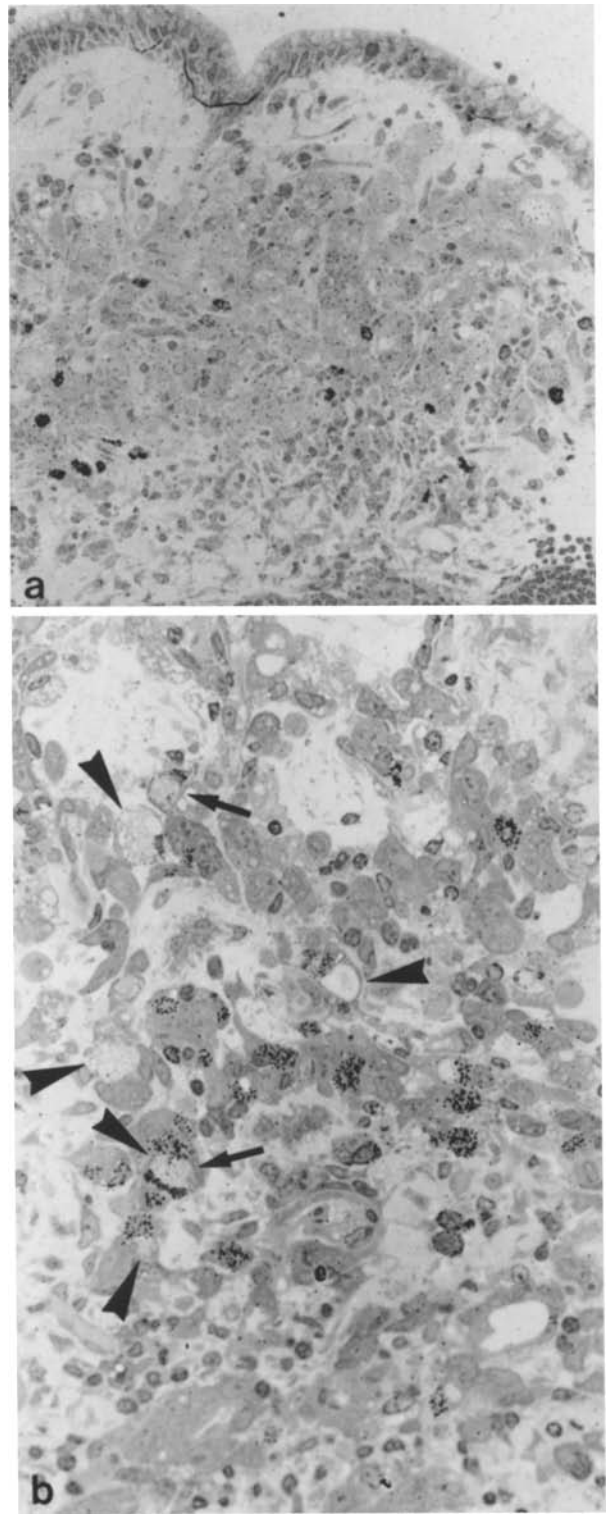
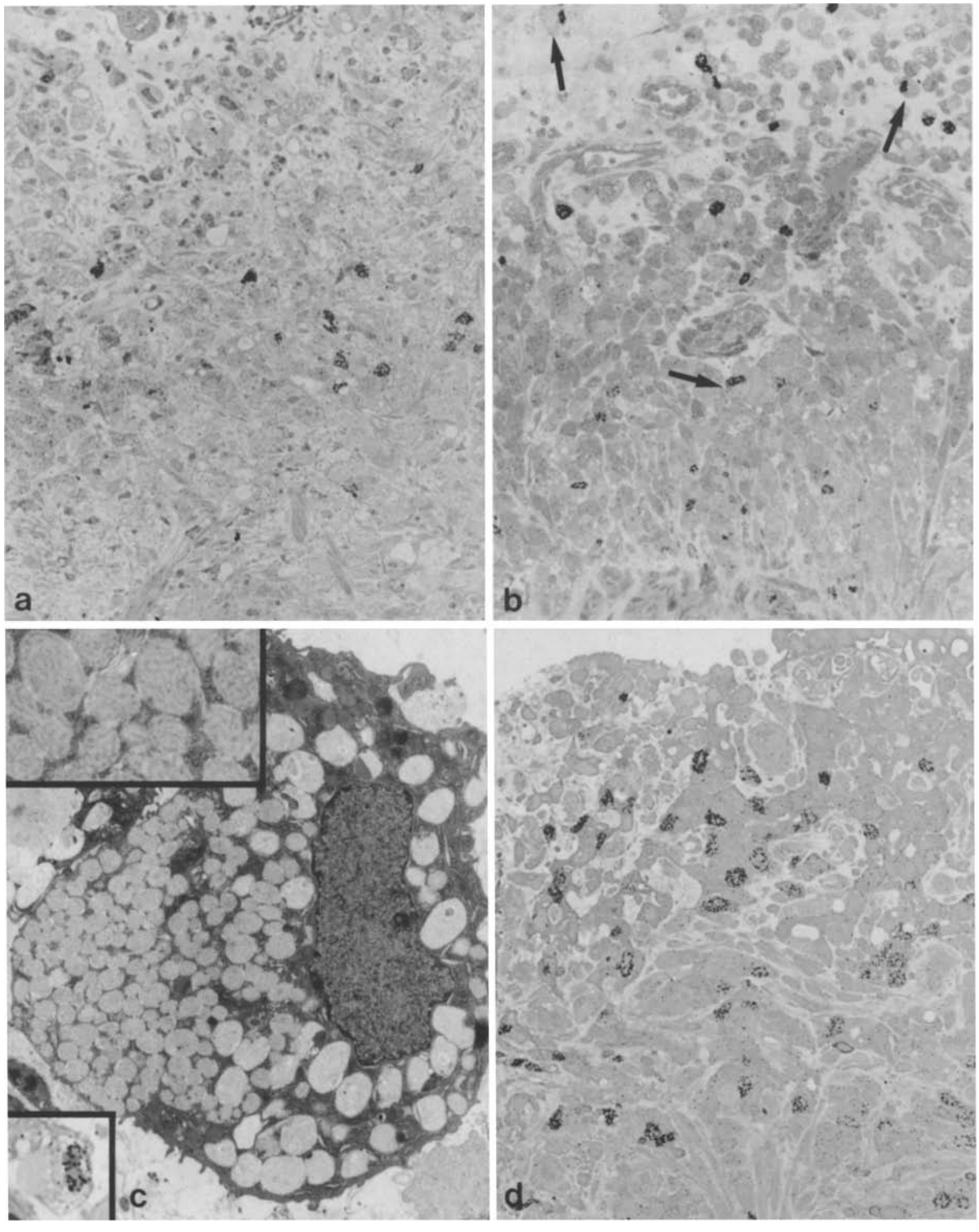


Fig. 7a, b. Autoradiographs of superficially spreading type intramucosal cancers. **a** The layered structure (case No. 15). Toluidine blue. ×200. **b** A disorganized layered structure (case No. 21), in which proliferating signet ring cells (*arrows*) and intestinal type cells (*arrow heads*) are randomly distributed. Toluidine blue. ×345



(Fig. 8a–d)

In 3 of the 8 cancers of the superficially spreading type, the autoradiographically-defined layered structures were found (Fig. 7a). In several parts of all the 8 cases, however, the small-cell layer at the middle level was indistinct, although signet ring cells of the foveolar type and the glandular type tended to be distributed in the superficial and the deep part of the mucosa, respectively. The signet ring cells of the foveolar-type were labeled with ^{3}H-thymidine in 6 of the 8 cases. The intestinal-type cells were commonly seen and randomly distributed in the mucosal lesion (Fig. 7b).

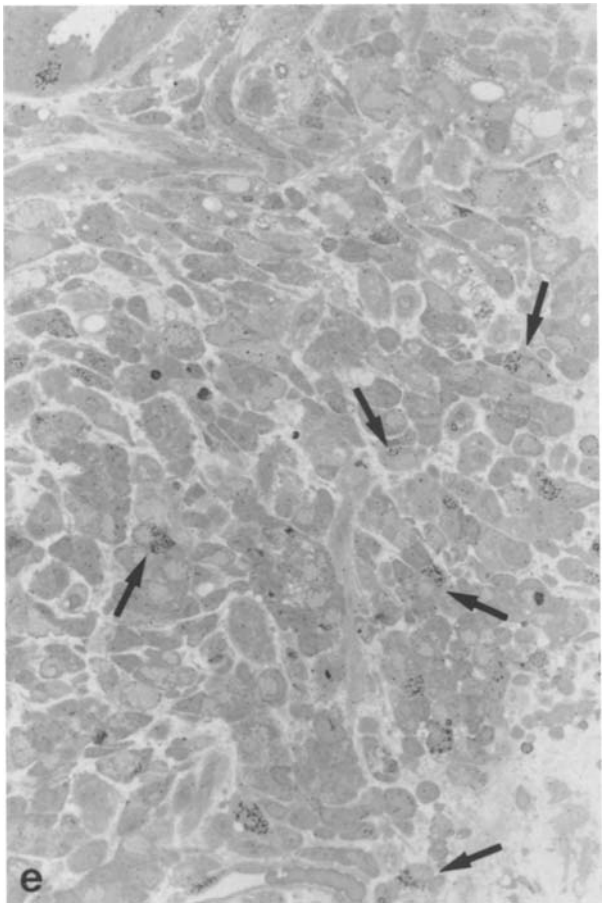


Fig. 8a, b. Autoradiographs of advanced signet ring cell carcinomas with the mucosal spread larger than 2 cm in diameter. Toluidine blue. ×250. **a** The layered structure (case No. 30). **b** A disorganized layered structure (case No. 28) with labeled signet ring cells (*arrows*). **c** A foveolar-type signet ring cell in an advanced cancer (case No. 28) labeled in corresponding autoradiograph of the adjacent semithin section (*lower inset*). The upper inset is higher magnification of the mucous granules. ×5000. Total cross-sectional area of mucin is 65.6 μm^2. **d, e** Autoradiographs of advanced signet ring cell carcinomas with the mucosal spread smaller than 2 cm in diameter. Labeled signet ring cells (arrows) and small round cells are randomly distributed in the mucosa. **d** case No. 25. Toluidine blue. ×250. **e** case No. 23. Toluidine blue. ×350

In the advanced cancers with a diameter of mucosal lesion larger than 2 cm, the autoradiographically-defined layered structure was seen in several parts of the lesions in the cases, No. 27 and No. 30 (Fig. 8a). But in large part of the lesion, intestinal-type cancer cells and proliferating signet ring cells (foveolar type, Fig. 8c) were observed quite frequently and small round cells were distributed throughout the depth of the mucosa (Fig. 8b).

In the advanced cancers with the mucosal spread smaller than 2 cm in diameter, the layered structure was scarcely seen. Small round cells and proliferating signet ring cells were often arranged in cords and were randomly distributed in the mucosal lesion (Fig. 8d, e). The intestinal-type cancer cells were not seen, except for the case No. 22.

Discussion

Signet ring cell carcinoma is thought to arise in the gastric mucosa not involved in intestinal metaplasia (Sugano et al. 1984). In the normal gastric mucosa, proliferating cells are confined to the glandular neck region, forming a generative cell zone (Hattori and Fujita 1976), and it is in this zone that signet ring cell carcinomas are thought to arise. Accordingly, it was reported that the earliest signet ring cell carcinomas were found in the lamina propria at the gland-neck level (Taki and Kuwabara 1981). In these minute cancers, a layered structure is generally seen, in which signet ring cells in the superficial and the deep layer are produced by division of the small round cells in the middle layer (Sugihara et al. 1985). This may imply that in an early stage of cancer progression cell proliferation and differentiation in signet ring cell carcinomas may be still dependent upon the microenvironments of the generative cell zone.

In order to study the significance of the layered structure in the progression of signet ring cell carcinomas, we investigated the occurrence of the layered structure in intramucosal and advanced cancers. From autoradiographic findings, we defined the layered structure to have the middle layer composed of proliferating small cells and the superficial layer of non-labeled signet ring cells, irrespective of the presence of the deep layer. The deep layer was not always found, and it appeared not related to growing patterns of the tumours. To describe cell differentiation in the tumours, we classified the signet ring cells into 3 types: a foveolar, a glandular and an intestinal type, from fine-structural characteristics of the mucous granules and microvilli, and from mucin-histochemistry. Each cell type in our classification corresponded to the normal counterpart not only cytologically but also in the pattern of distribution in the mucosa; the foveolar-type and the glandular-type cancer cells were distributed at the superficial and the deep part of the mucosa, respectively, as the foveolar and the glandular cells in the normal mucosa. The intestinal-type cancer cells were distributed randomly in the mucosal lesion. Our foveolar type and glandular type correspond to an eosinophilic type by Oota and Sobin (1977) and our intestinal type conforms to a goblet cell type by them. Although they described the cells with microcysts to constitute a distinct cell type, the present study

showed that the cells with microcysts can be further classified into those 3 cell types described above.

In the intramucosal cancers smaller than 4 cm in diameter, the layered structure was often seen. The typical layered structure tended to be seen in the peripheral part of the lesion, where the normal stromal architecture of the mucosa seems to be fairly preserved. In these cancers, only a few intestinal-type cells were seen. Most of the signet ring cells in the superficial and the deep layers lacked proliferative activity and they may be terminally differentiated postmitotic cells. These have been reported in several cancers (Pierce and Wallace 1971; Wylie et al. 1973).

In the large intramucosal cancers, the cancer cells of the foveolar and the glandular type retained a tendency to be distributed in the superficial and the deep part of the mucosa, respectively. However, the middle, generative cell layer was often indistinct in autoradiographs; the proliferative, small round cells were randomly distributed even in the superficial and the deep layers, and foveolar-type signet ring cells were occasionally proliferative. These changes seemed to be caused by atrophy of the mucosa resulting from repeated mechanical and chemical injuries on the mucosa bearing the tumour, possibly reflecting a disorganization of stromal structures which may support microenvironments of the mucosa. In the large intramucosal cancers, the intestinal-type cells were commonly encountered. It seems unlikely that the occurrence of the intestinal-type cells in the cancers merely reflects the metaplastic changes in the surrounding mucosa as they occurred independently of the degree of the intestinal metaplasia in the surrounding mucosa. Rather, it seems to be related to a kind of intestinal metaplasia of the tumour cells. Intestinal phenotypes were described in advanced signet ring cell carcinomas by Sasano et al. (1969) and Nevalainen and Jarvi (1977). Tatematsu et al. (1984) reported that intestinal-type cells occur time-dependently in experimental gastric adenocarcinomas. Thus, dissociation of the middle generative layer and appearance of the intestinal-type cells seem to be a time dependent phenomenon. In large mucosal cancers, disarrangement of the layered structure may reflect a slow growth of the tumours rather than an enhanced tumour invasiveness.

The mucosal lesion in the advanced cancers larger than 2 cm in diameter often showed a disorganized layered structure as in the large intramucosal cancers, and the layered structure was only partially seen. These advanced cancers may have developed slowly from the early cancers with the layered structure. But, in the advanced cancers with a mucosal lesion smaller than 2 cm in diameter, the layered structure was rarely seen, and except for one case, intestinal-type cells were not found. The proliferative cells were both small round cells and signet ring cells randomly distributed throughout the depth of the mucosa. These cancers seem to start without the layered structure, growing independently of the microenvironments of the mucosa. Absence of intestinal-type cells suggests that the change may have occurred not long after they developed. These findings may suggest that tumours starting without the layered structure may be highly invasive and readily become an advanced cancer. In a case studied in a separate paper, we have encountered a minute signet ring cell carcinoma without the layered structure, which had already invaded the submucosa (Hattori et al. 1986).

From these findings, it can be postulated that there may be different kinds of signet ring cell carcinomas, which disclose slow and rapid growth patterns. Difference between these growth patterns may be closely related to the presence or absence of the layered structure in early stages of tumour development. The discrimination of the different kinds in signet ring cell carcinomas is supported by our recent studies on DNA ploidy patterns using the present cases; the intramucosal cancers showed diploidy irrespective of tumour size, whereas the small advanced cancers without the layered structure showed aneuploidy or mixture of aneuploidy and diploidy (unpublished data). The materials studied in the present paper were limited to intramucosal (surface spreading) and small advanced (deeply infiltrative or penetrating) cancers, but there are transition forms between these growth patterns. Whether or not the growth pattern may alter during the tumour development is to be clarified in further studies.

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