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Neoplastic Paneth Cells in the Experimental Murine Carcinoma of the Small Intestine

Woo Ho Kim, M.D., and Yong Il Kim, M.D.

Department of Pathology, Seoul National University College of Medicine, Seoul, Korea.

The purpose of this study is to elucidate the participation of Paneth cells in experimentally induced adenocarcinoma of the intestine. The rats were fed with N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) dissolved in drinking water ad libitum at a concentration of 100μg/ml for 28 weeks. They were sacrificed 12 weeks after the last MNNG administration. A number of tumor cells containing large eosinophilic granules in their supranuclear cytoplasm (Paneth cells) were observed in about 20% of the experimentally induced adenocarcinoma of the small intestine. The granules were stained positively with Lendrum, periodic acid-Schiff, Masson's trichrome, and Mallory's phosphotungstic acid hematoxylin. Ultrastructurally, the granules were round, osmiophilic, and relatively even in size. We compared the morphologic features of the Paneth cell-containing small intestinal adenocarcinomas (Group I) with those without Paneth cells (Group II). Group I was distinguished from Group II by its better differentiation, larger tumor size and lower incidence of calcification. Although Paneth cells are extremely rare in human gastronitestinal carcinomas, twenty percent of MNNG-induced intestinal carcinomas harbor Paneth cells. The neoplastic Paneth cells in experimantal carcinomas may differentiate from uncomitted cells in the deeper portion of the crypt.

Key Words: *Paneth cells, Experimental carcinogenesis, N-methyl-N'-nitro-N-nitrosogunanidine, Small intestinal carcinoma*

INTRODUCTION

Paneth cells are the normal constituents of the small and large intestinal mucosa in humans and certain mammals including rats. They are located in the deep portion of the crypt. Although the occurrence of those cells in malignant tumors has been thought to be extremely unusual in both humans (Holmes, 1965) and experimental animals, several studies confirmed their existence. Stern and Sobel (1961) demonstrated Paneth cells in small intestinal adenocarcinomas, and Schartfenberg and DeCamp (1975) reported the appearance of Paneth cells in carcinomas arising in Meckel's diverticulum. Similarly, neoplastic Paneth cells have been described in duodenal carcinomas (Miyajima and Takeuchi, 1979) and in gastric adenocarcinomas as well (Heitz and Wegmann, 1980). A gastric adenoma, of which 95% of tumor cells were Paneth cells, was reported recently (Rubio, 1989).

Existence of neoplastic Paneth cells in experiemental tumors was confirmed in mice and in rats (Dunn and Kessel, 1945), and Stewart and Lorenz (1947) described the existence of Paneth cells in both precancerous and overt carcinomas of the small intestine in mice. Nevertheless, the significance of Paneth cell-containing tumors has remained uncertain. In this study, we attempted to elucidate the nature of the experimentally-induced gastrointestinal tumors, and to characterize the morphologic features of neoplastic Paneth cells as well as to identify the differences between the tumors with and without Paneth cells.

Address for correspondence to: *Yong Il Kim, M.D., Department of Pathology, Seoul National University College of Medicine, 28 Yongon-dong, Chongno-gu, Seoul 110-460, Korea (Tel: (02) 760 -2550)*

MATERIALS AND METHODS

A total of 233 Sprague-Dawley rats were used for this study. The animals were administered N-methyl-

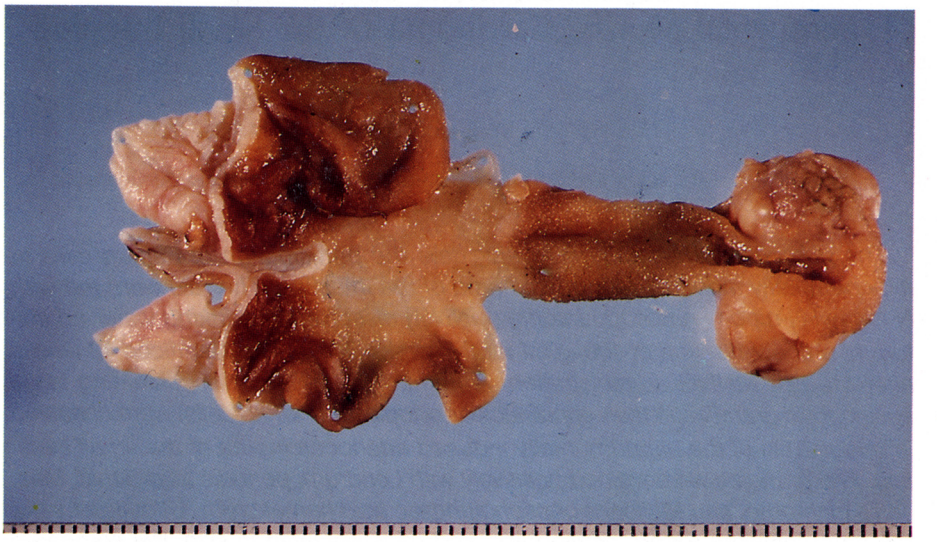

Fig. 1. Gross photograph of gastro-duodenal mucosa. There is a small encircling, exophytic adenocarcinoma at the duodenum, 2.0cm apart from pyloric ring.

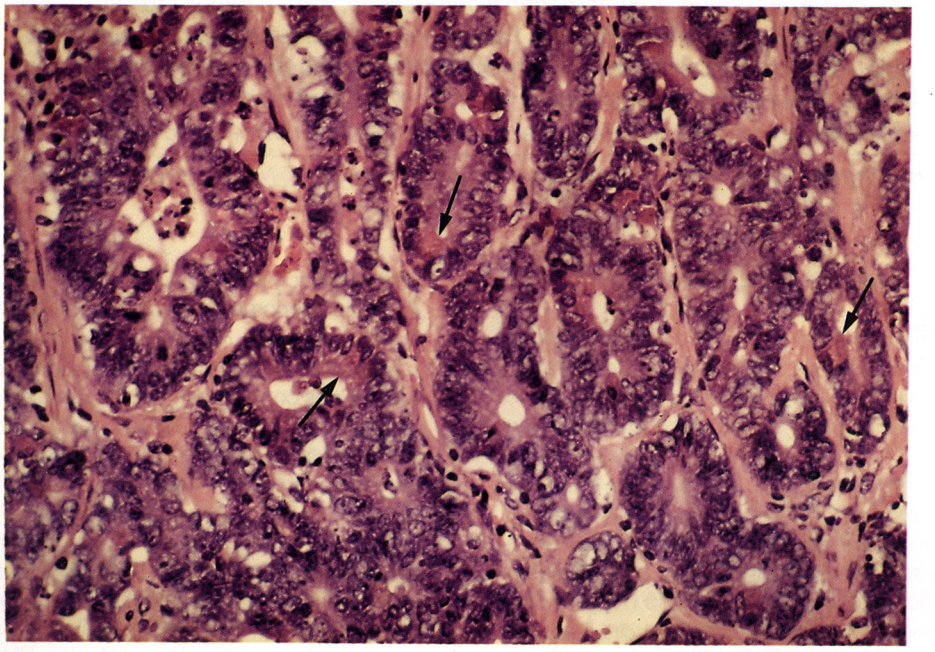

Fig. 2. Photomicrograph of Paneth cell positive adenocarcinoma. Neoplastic Paneth cells (arrows) are admixed in a well differentiated adenocarcinoma of the small intestine (HE, X200).

N'-nitro-N-nitrosoguanidine, a potent carcinogen, which was diluted in drinking water at a concentration of 100μg/ml and fed ad libitum for 28 weeks. The animals were sacrificed 12 weeks afters cessation of MNNG administration. A detailed description had been made in the previous report (Han et al., 1985; Kim et al., 1987a). The stomach and duodenum were opened along the greater curvature and immediately fixed in 10% neutral formalin. The fixed stomach and duodenum were serially cut along its longitude in the width

of 0.3cm. All sections, including the detected tumors, were processed for paraffin embedding, and a 5μ-thick section with hematoxylin-eosin stain was made. Periodic acid-Schiff (PAS)-alcian blue, Masson's trichrome, phosphotungstic acid hematoxylin (PTAH) and Lendrum stains were obtained if needed.

For electron microscopic investigation, paraffin blocks from four tumors were deparaffinized, post-fixed with osmium tetraoxide, and a double staining by lead citrate and uranyl acetate was applied.

The morphologic parameters of the tumors, such as degree of differentiation, depth of tumor invasion, tumor size, predominant cell component, squamous metaplasia and tumor necrosis, were scored in each tumor and analyzed with Yates' correction of chi-square test.

RESULT

Among the 233 MNNG-administered rats, 80 developed malignant tumors. The number of tumors found in each rat ranged from one to six, accounting for a total of 124 tumors in 80 animals; they were gastric carcinomas (62), small intestinal carcinomas (50), and sarcomas (12) in gastrointestinal tracts. Fifty small intestinal carcinomas were detected in 49 rats, of which 10 small intestinal carcinomas in 10 rats contained Paneth cells as their components. None of the 62 gastric carcinomas disclosed Paneth cells (Table 1).

The neoplastic Paneth cells were characterized by supranuclear refractile eosinophilic granules. Their

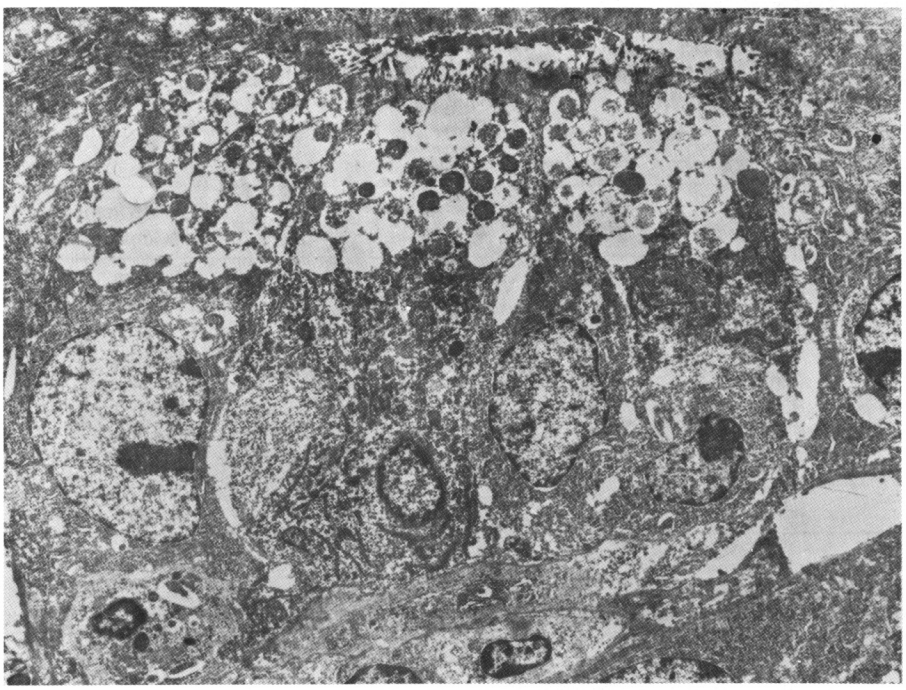

Fig. 3. Several Paneth cells are located in a row. They have irregular nuclei and prominent nucleoli. Surface microvilli are prominent (×5,400)

Table 1. Paneth cell differentiation rates in MNNG-induced gastric and intestinal carcinomas.

	Paneth cell present	Paneth cell absent	Total
Gastric carcinoma	0	62	62
Small Intestinal carcinoma	10	40	50

nuclei were large, irregular, and hyperchromatic, but otherwise were not morphologically distinguishable from those of other constituent cells of the tumors. The granules were stained bright red in Lendrum, red in PAS, dark red in Masson's trichrome, and light brown in PTAH. These staining characteristics were identical with those of Paneth cells in the adjacent normal intestinal crypt.

Semithin sections of the Epon-embedded tissue stained with toluidine blue revealed blue granules in the cytoplasms of many tumor cells. Ultrastructurally, those granules were round, homogeneously granular, but showed varying intensities of osmiophilia. The limiting membrane was not identified. Their sizes ranged from 0.2-0.7μm in profile diameter. In the subnuclear and supranuclear portions, rough endoplasmic reticulum was well developed. On the apical surface of the Paneth cells, short microvilli were regularly developed.

We compared the following pathologic parameters of the small intestinal carcinomas containing Paneth cells (Group I) with those without Paneth cells (Group II): such as the size of the tumor, depth of invasion, mucin content, desmoplasia, neutrophilic infiltration, calcification and/or ossification, squamous metaplasia, and necrosis. Among the above variables, the sizes of the tumors and calcification showed the highest chi-square (Table 3). The mean diameters of Groups I and II tumors were 14.3 mm and 18.5 mm, respectively. While 25% of the Group II tumors were larger than 20mm in diameter, 50% of Group I tumors exceeded the above diameter. The calcification and/or ossification of the tumors were more frequent in Group II (35%) than Group I (10%). However, they were not statistically significant due to the limited number of cases.

The degree of differentiation of the tumors seemed to be significantly different (Table 4). All of the Group II tumors were poorly differentiated or undifferentiated.

Table 2. Morphologic characteristics fo Paneth cell positive small intestinal carcinomas

Case No.	Tumor size	No. of PC/HPF	Degree of differentiation	Distance from pylorus	Depth of invasion
1	3mm	10.2	Well diff.	4mm	mucosa
2	8mm	0.1	Mod. diff.	10mm	subserosa
3	11mm	11.8	Well diff.	50mm	serosa
4	12mm	7.1	Mod. diff.	90mm	serosa
5	20mm	10.3	Mod. diff.	100mm<	serosa
6	20mm	5.1	Well diff	100mm<	serosa
7	23mm	5.1	Mod. diff	100mm<	serosa
8	25mm	6.3	Mod. diff.	30mm	serosa
9	40mm	6.3	Mod. diff.	100mm<	serosa
10	45mm	2.1	Mod. diff.	15mm	serosa

Table 3. Prevalence and statistical significance of morphologic variables comparing Paneth cell positive to Paneth cell negative small intestinal tumors

Variables	Prevalence	Chi-square	Significance
Size of tumor (larger than 20mm)	0.30	2.38	N.S.
Calcification	0.30	2.38	N.S
Desmoplasia	0.56	1.30	N.S.
Depth of invasion (s or ss)	0.94	0.35	N.S.
Necrosis of tumor	0.48	0.32	N.S.
Mucinous component	0.44	0.18	N.S.
Neutrophilic infiltration	0.44	0.08	N.S.
Squamous metaplasia	0.35	0.00	N.S.

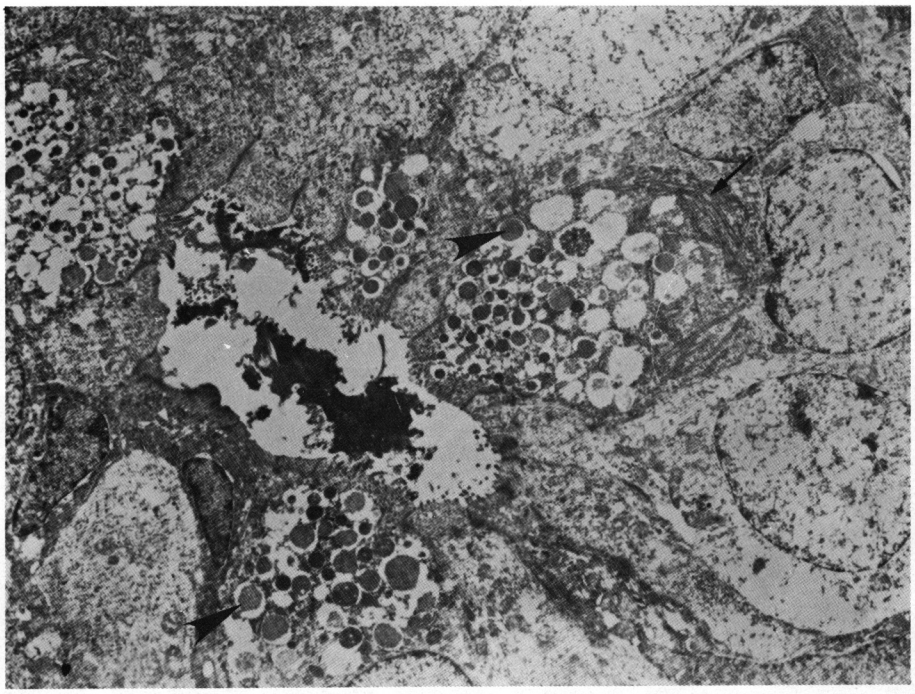

Fig. 4. Electron microscopic picture of intestinal tumor. There are several Paneth cells around the lumen. Note the variable sized granules (arrow heads), well developed ER (arrows) and microvilli at the surface (×5,400).

Table 4. Degree of differentiation of group I and group II carcinomas

	Well or mod. differentiated	Poorly or undifferentiated	Total
Paneth cell present	10	0	10
Paneth cell absent	32	8	40
Total	42	8	50

DISCUSSION

MNNG is a well-known potent gastrointestinal carcinogenic agent (Sugimura and Fujimura, 1965) by which a majority of the tumors develop in the gastric mucosa and the upper part of the small intestine, especially of the duodenal mucosa around periampullary area. The histologic similarity between human carcinomas and MNNG-induced cancers has drawn attention from many observers. The histologic features of the MNNG-induced gastric tumors have been well-described (Baralow et al., 1970; Park et al., 1980) in the literature, However, intestinal tumors have not been precisely described despite the fact that their histologic characters differ from gastric tumors. Most of the gastric tumors are well or moderately differentiated adenocarcinomas, and a minority includes signet ring cell carcinoma as a tumor component (Kim et al., 1987b). Small intestinal carcinomas are also well or moderately differentiated, but they show deeper invasion, frequent calcification of ossification, scanty lymphocytic aggregation, and abundant mucin components.

To be neoplastic, the Paneth cells must be an integral part of the tumor tissue (Lewin, 1968). All of our cases fulfilled this condition. Moreover, the neoplastic Paneth cells in our cases disclosed large, irregular and hyperchromatic nuclei in electron microscopic pictures.

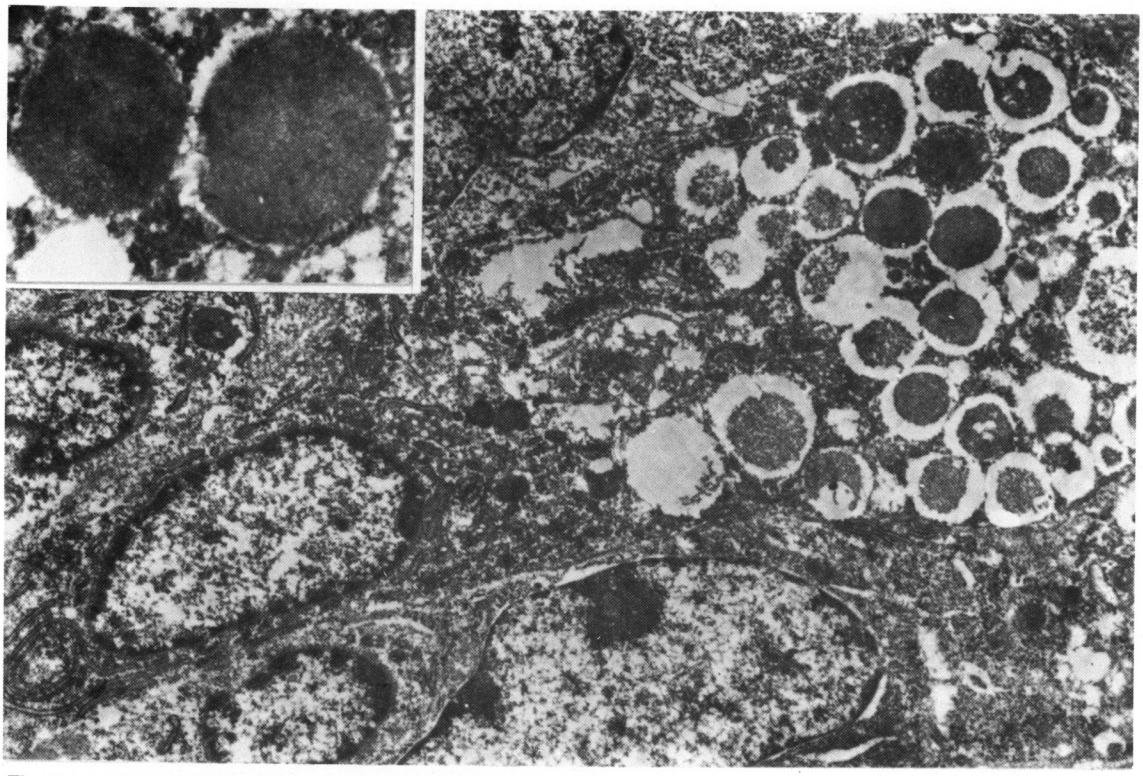

Fig. 5. A Paneth cell demonstrating abundant supranuclear dense granules and basally located rough ER. (×10,000) Inset shows homogeneous nature of granular content (×35,000).

One of our interests was whether there were any biologic differences between Group I and Group II tumors. The carcinomas in Group I had several distinguishing features in comparison with those of Group II: better differentiation, larger tumor size, and absence of calcification.

Among 50 small intestinal carcinomas, eight were poorly differentiated or undifferentiated carcinomas, and none of them contained Paneth cells in their elements (Table 4). This finding may imply that the presence of Paneth cells in the small intestinal carcinomas reflects better differentiation.

The tumors with calcification/ossification were larger than the tumors without (22.3mm vs. 12.1mm, $p < 0.05$), and the tumors with Paneth cells were larger than the tumor without. However, Paneth cell positive tumors rarely showed calcification.

The rarity of Paneth cells in human gastrointestinal tract cancer has not been fully understood, but could be best explained by the following suggestions. Firstly, the Paneth cell is a highly specailized cellular component of small intestinal mucosa and can not be appreciable in the conventional adenocarcinoma. On the contrary, Paneth cells are frequently seen in tubular adenomas of the stomach and gallbladder which have been known to arise in the background of intestinal metaplasia. Our data indicates that only some forms of intestinal adenocarcinomas, when differentiated, discloses Paneth cell differentiation. Secondly, many intestinal carcinomas develop from the surface epithelia or mucous neck glands which are devoid of the potential for Paneth cell differentiation, while the tumors arising from the indifferent cells within the deeper portion of the crypt (replication zone) can express their potenial for multidirectional differentiation.

It is plausible that when intestinal mucosa is exposed to a potent carcinogen such as MNNG, carcinomas develop through the two different histogenesis: (1) some carcinoma arise from foveolar epithelium or mucous neck cells, and they are devoid of Paneth cells regardless of their cellular differentiation; (2) others develop from indifferent cells (pleuripotential cells) in the replication zone. Thus, the tumors from both lines can be well-differentiated or poorly differentiated and a minor portion of the well differentiated adenocarcinomas from latter group may differentiate into Paneth cell

line.

Other parameters such as degree of desmoplasia, depth of invasion, extent of tumor necrosis, mucin secreting activity, neutrophilic infiltration or squamous metaplasia were not significantly different in both groups.

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