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Signet Ring Cell Carcinoma of the Stomach in N-methyl-N'-nitro-N-nitrosoguanidine-Administered Rats. Report of Two Cases with Special Reference to Its Histogenesis

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= **Abstract** =Sprague-Dawley rats, orally administered with N-methyl-N'-nitro-N-nitrosoguanidine (100 μg/ml dissolved in drinking water given ad libitum) for 28 weeks were sacrificed 12 weeks after the last administration. Of 89 neoplastic lesions from 96 rats, two rats developed signet ring cell carcinoma (SRC) with focal mixture of poorly differentiated adenocarcinoma in one, involving the full thickness of the gastric wall and blood vessels. Aside from the incipient atypical cell growth along the foveolar epithelium there was a histochemical similarity of mucin between SRC and atypical foveolar epithelial cells. These observations suggest that SRC develops from non-metaplastic foveolar epithelium of the stomach.

Key Words: ***Experimental gastric carcinoma, Signet ring cell carcinoma, N-methyl-N'-nitro-N-nitrosoguanidine***

INTRODUCTION

Experimental induction of adenocarcinoma of the stomach has been rarely successful until the late 1960's when Schoental (1966) and Sugimura *et al.* (1967, 1969) were first able to produce stomach cancer with highly selectivity by administation of N-methyl-N'-nitro-N-nitrosoguanidine (MNNG). Thereafter, substantial reports on the MNNG-induced gastric carcinoma have been published,and provided a considerable amount of information in the understanding of human gastric carcinogenesis. As for the histologic type, most of the MNNG-induced gastric carcinoma are either well or moderately differentiated adenocarcinomas (Sugimura *et al.* 1969; Park *et al.* 1980), and less differentiated tumors of the rat stomach were only induced by adding surfactants (Takahashi *et al,* 1975) and gastrin (Tahara and Haizuka 1975) to MNNG.

Signet ring cell carcinoma (SRC) of the human stomach is known as a variant of adenocarcinoma with different growth patterns and biological behavior from others, which suggests that they have an unique carcinogenesis. However, experimentally induced SRC of the rat stomach was seldom referred in literature to back up its human counterpart, although there are random cases of N-nitrosobutylurea (NBU)-induced or MNNG-induced SRC in dogs (Watanabe *et al.* 1979a & b).

We had examined eighty nine MNNG-administered rats harboring varieties of gastric and small intestinal tumors, among which were only two carcinomas of SRC type. The purpose of this report is to abstract the possible carcinogenesis of experimentally induced gastric SRC of the rats induced by MNNG.

MATERIALS AND METHODS

One hundred and twenty days old Sprague-Dawley rats, weighting 130-150 g in body weight at the beginning of the experiment were used. They were administered 100 μg/ml MNNG dissolved in the drinking water ad libitum for 28 weeks. The mean of the total amount of MNNG administered to each

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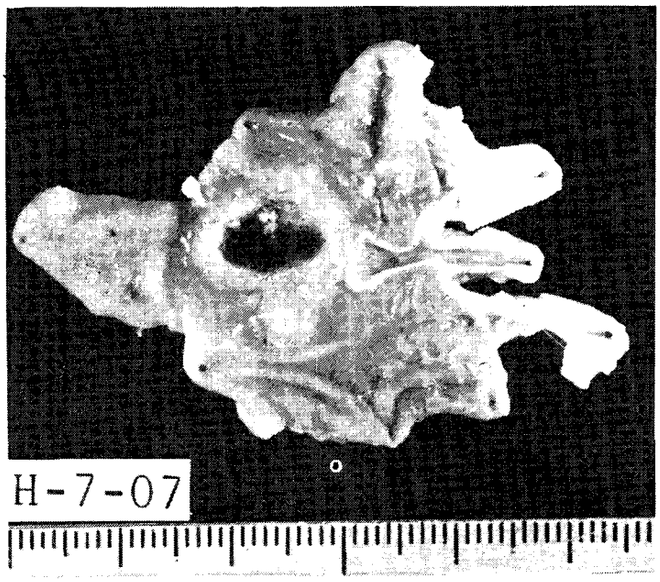

Fig. 1. Gross picture of case 1. There is an ulcerofungating tumor along the lesser curvature of glandular stomach.

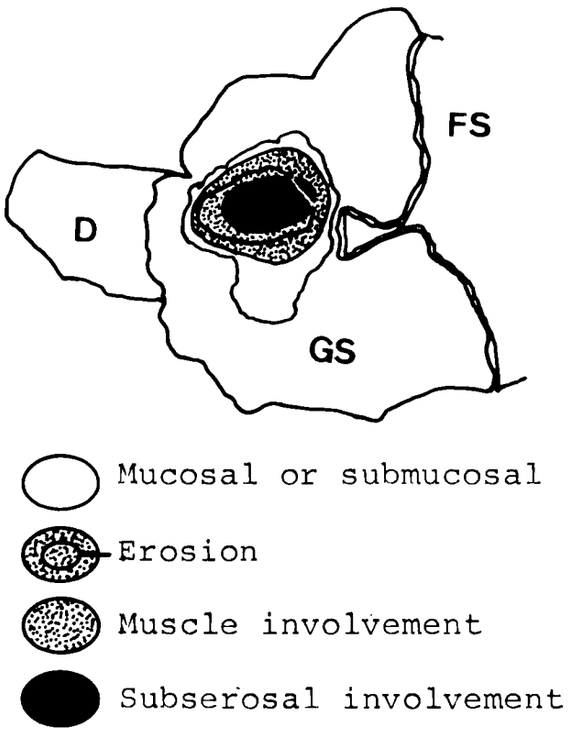

Fig. 2. Schematic diagram of case 2. D:duodenum, GS:glandular stomach, FS:forestomach

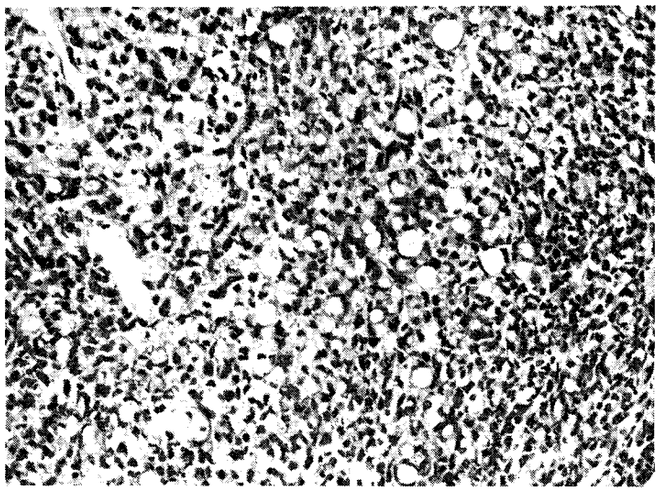

Fig. 3. A Photomicrograph of a portion of signet ring cell carcinoma. (HE, x200)

animal was 617.4 mg (2.9-3.3 mg/day). Animals were sacrificed 12 weeks after the last dose. The stomach was removed and immediately opened along the greater curvature, pinned to a cork plate and fixed in 10% neutral formalin solution. Major organs other than the stomach were examined grossly. The stomachs were serially sectioned with a width of 3 mm and processed for paraffin embedding,and all of the blocks were sectioned for histotopographic reconstruction. Hematoxylin-eosin, periodic acid-Schiff, colloidal iron and alcian blue (pH 2.5 and 1.0) stainings were performed.

CASES

Case 1. (H-7-07)

The glandular stomach was 1.7 cm in length along the lesser curvature. There was a large excavating and ulcero-fungating lesion (Borrmann III) along the lesser curvature of the glandular stomach (Fig. 1). The lesion, 1.7 x 1.5 cm, had a central ulceration, 0.9 x 0.6 cm, which was coated by dark necrotic material (Fig. 2). Microscopic examination showed that the tumor was almost entirely composed of SRC cells in varying stages of maturation. All of the SRC cells were strongly PAS positive, moderately positive to alcian blue staining (pH 2.5) and negative to both colloidal iron and alcian blue in pH 1.0 (Fig. 3). The tumor extended into the proper muscle and subserosal layer, and the SRC cell population was much heavier at the deeper portion of the tumor. The proper muscle layer was fragmented and embedded partly within the tumor mass (Fig. 4). Many meduim sized vessels were plugged with tumor emboli of SRCs (Fig. 5). At the marginal portion of the tumor tissue, individual SRC cells were scattered in the lamina propria (Fig. 6), and a few glands near the tumor were partialy lined

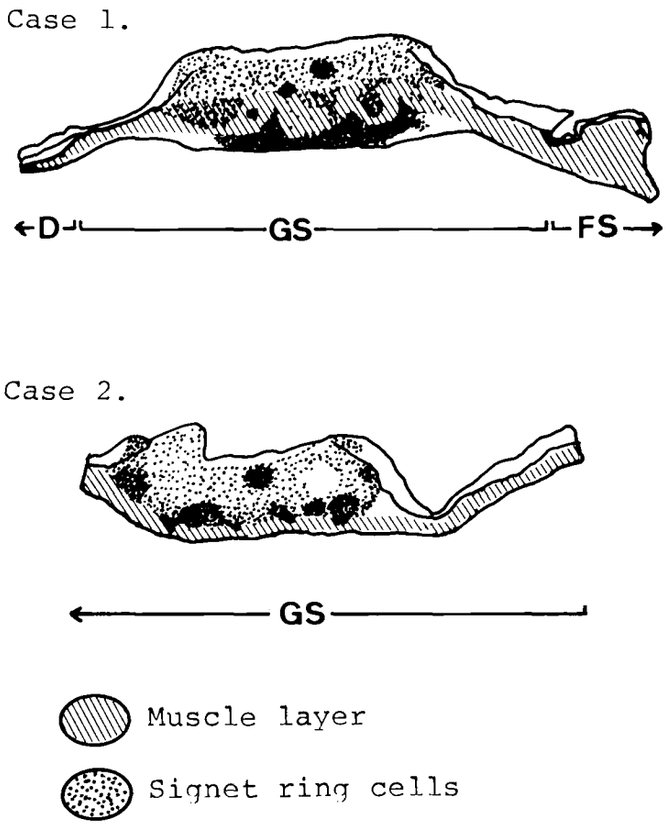

Fig. 4. Schematic diagram of cut surface of case 1 and 2. D, GS and FS:same as figure 2.

Fig. 6. The individual signet ring cells are scattered in *lamina propria, and the transition from foveolar* epithelium to signet ring cells is evident. (HE, x100)

by a single layer of SRC. Glandular configuration of the SRC which resembled pyloric glands were noted (Fig. 7). The background mucosa of the

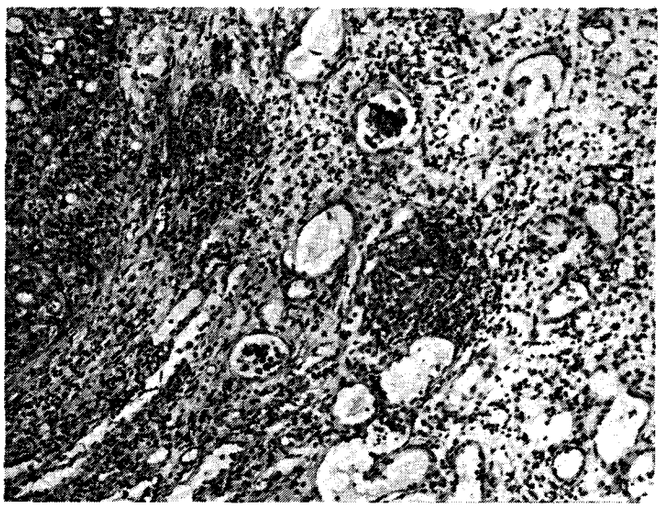

Fig. 5. Tumor emboli composed of signet ring cells are seen in the dilated venules. (HE, x100)

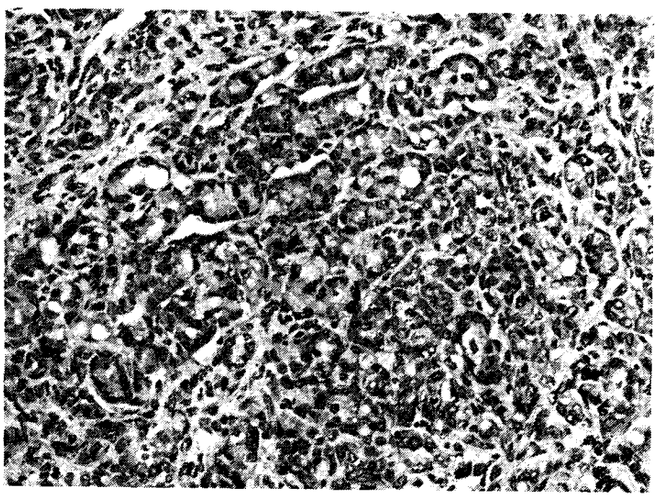

Fig. 7. Some of the tumor cells resemble the pyloric gland epithelium. (HE, x200)

glandular stomach showed no evidence of intestinal metaplasia.

Cases 2 (L-4-14)

The glandular stomach measured 1.8 cm along the lesser curvature, and contained an ulcero-infiltrative tumor lesion of 2.0 x 2.0 cm size at the anterior wall of the glandular stomach, which was histologically SRC similarly shown in Case 1. There was also another polypoid carcinoma measuring 0.5 cm in diameter at the the duodenum, 4.5 cm distal to the pyloric ring. The latter tumor was proven to be a moderately differentiated adenocarcinoma of the duodenum with marked desmoplasia

and heavy neutrophilic infiltration. The gastric tumor reached to the outer portion of the muscle coat (Fig. 4) and the duodenal lesion extended to the serosal layer. The histochemical characteristics of the SRC were identical to case 1; PAS strong positive, alcian blue (pH 2.5) weakly positive, and colloidal iron negative. The background mucosa was totally free of intestinal metaplasia.

DISCUSSION

Human gastric carcinogenesis is rather complex and categorized into two basic types; the one develops from the preceeding phase of intestinal metaplastic change and the other is of de novo gastric mucosal origin (Lauren 1965). Over two thirds of gastric carcinomas are assured to arise in the stomach with intestinal metaplasia, and thus the differentiated tubular or papillary adenocarcinoma of the intestinal type comprises the majority, whereas the others including SRC occur in the non-metaplastic mucosa of the human stomach. Despite the facts that SRC is not a rare type and approximately 35.5% of human gastric carcinomas are predominantly composed of SRCs and the other 18.7% include SRC as a minor component even in early gastric carcinomas (Kim *et al.* 1986), there still remains many questions and controversy on its origin of cell and the clinical significance. Thus, the experimental induction of SRC has been expected to answer the above question.

Our two cases seem to meet the general criteria of experimental carcinomas based on that both involve the full thickness of the stomach wall along with apparent vascular invasions. And their staining properties are the same with those in human SRC. The aggregated tumor cells in case 1 resembled histologically the pyloric gland cells by their microalveolar arrangement, abundant pale pinkish cytoplasms and basally located nuclei. However, their staining characteristics were different; SRC are strong PAS positive, whereas the pyloric gland are negative. Rather, SRC was similar to that of the foveolar epithelium in the aspect of histochemical features; strong PAS positive, moderate alcian-blue (pH 2.5) positive and colloidal iron negative. Those features were frequently seen in the early stage of signet ring cell tumors and was described as "party wall appearance" (Yamashina 1986). Moreover, a few foveolar epithelial linings near the tumor contained discrete cells which are identical to SRC. These observations, together with the fact that adjacent mucosa was devoid of metaplastic change, seem to suggest foveolar epithelium as a precursor of SRC variant.

Similar histologic and histochemical findings were described in two dogs administered with N-nitrobutylurea by Watanabe *et al.* (1979a & b). One dog demonstrated an incipient phase of intraglandular atypical cell growth with the same histochemical nature to SRC cells in the adjacent overt carcinoma in the pyloric portion. Another dog showed a histochemical change of the surface epithelial cells identical to that of adjacent SRC cells. Several foci of moderately differentiated adenocarcinoma were admixed in the first case and this is not an uncommon feature in human cases as well. Of the following two possibilities raised to explain this mixture, a single precursor cell theory of bidirectional differentiation into SRC and gland-forming adenocarcinoma seems more feasible than multiple primary foci of various histologic types which conglomerate to develop the mixed lesion (Ming 1977). In human stomachs the two components are usually mixed randomly and the transition between the two components is frequently observed. However, this is not a simple question that can be easily solved, and requires further clarification by repeated experimental study.

There have been no proven factors postulated to determine the histologic type of the gastric carcinomas. With our previous experimental works on MNNG-induced gastric carcinogenesis, we have felt that the replication zone of the gastric mucosa is much vulnerable and becomes the main site of neoplastic transformation by a carcinogenic exposure, and eventually to develop a tubular form of adenocarcinoma. However, when a foveolar epithelium is simultaneously exposured with the same carcinogen, a SRC may also develop in a small instance because of its limited absorption capacity unless the mucosa undergoes into the metaplastic change.

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= 국문초록 =

N-methyl-N'-nitro-N-nitrosoguanidine 투여에 의하여 발생한 흰쥐 위의 인환세포암종. 선와상피세포로부터의 조직발생을 시사하는 2 증례보고

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저자들은 96마리의 Sprague-Dawley계 흰쥐에 N-methyl-N'-nitro-N-nitrosoguanidine을 100 μg/ml로 희석하여 식수에 타서 28주간 투여하였고 12주후 도살하여 위에 발생한 89개의 종양성 병변을 병리학적으로 검색하였던 바, 이중 두마리에서 인환세포암종을 발견하였다. 두 증례 모두에서 인환세포는 정상 위와상피로 부터의 이행상을 나타내었으며 조직화학적으로 위와상피의 점액과 유사한 반응을 보였다.

저자들은 인환세포암이 실험적으로 유발된 보고가 극히 드물기 때문에 이를 보고하였으며, 이 유형은 장형화생을 거치지 않은 위와상피로 부터 발생할 것으로 추측하였다.