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Induction of Signet Ring Cell Carcinomas in X-Irradiated Hypocatalasemic Mice (C3H/C_s^b)

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The stomach region of hypocatalasemic mice of both sexes was X-irradiated once with a dose of 20 Gy. Thirteen months after the irradiation, 3 out of 13 (20%) males and 2 out of 9 (15%) females were observed to have developed signet ring cell carcinomas in the glandular stomach. This finding was statistically significant ($P<0.01$ in males and $P<0.05$ in females) compared to a total absence of similar tumors in the non-irradiated controls. Local invasion of malignant tumors into muscle and subserosal layers was observed, but no metastatic tumors were found in distant organs.

Key words: Signet ring cell carcinoma — X-irradiation — Hypocatalasemic mice

Poorly differentiated adenocarcinomas of the glandular stomach have been reported to be induced by various agents such as N-methyl-N′-nitro-N-nitrosoguanidine (MNNG)[2] following vagotomy,[1] N-ethyl-N′-nitro-N-nitrosoguanidine (ENNG),[2] ENNG plus gastrin[3] and N-nitrobutylurea (NBU)[4] in dogs. The incidence of poorly differentiated adenocarcinoma was also found to be increased by the combined administration of MNNG and various kinds of surfactant in rats.[5] Tahara and Haizuka[6] in addition reported an enhanced induction of scirrhous adenocarcinoma by the combined administration of MNNG and gastrin or serotonin. However, little is known about poorly differentiated gastric carcinomas in mice.[7] No good experimental models are yet available for the induction of signet ring cell carcinoma in mice. A model of poorly differentiated adenocarcinomas in mice would provide a new research tool for experimental gastric carcinogenesis. Previously we reported[8, 9] on the induction of well differentiated adenocarcinoma of the glandular stomach in ICR or CF_1 mice by X-irradiation. We have been maintaining a mutant of C3H mice, hypocatalasemic mice (C_s^b),[10] which have lower catalase and higher superoxide disumutase activity compared to those of the original C3H mice. This strain is of interest for cancer research as the mice are sensitive to tumor induction by hydrogen peroxide[11] and by radiation.[12] Therefore, we decided to examine the possibility of induction of signet ring cell carcinomas in the glandular stomach by localized X-irradiation in C_s^b.

C_s^b have been maintained under a sibling mating regime in our laboratory. Six-week-old mice were irradiated with a dose of 20 Gy of X-rays to the stomach according to the method described previously,[11, 12] and were maintained for 13 months until killed for autopsy. They were fed with commercial diet MF (Oriental Yeast) and provided with tap water *ad libitum*. The animals were maintained under the guidelines set forth in the "Guide for the Care and Use of Laboratory Animals" by Hiroshima University.

Sixty-five animals of both sexes were irradiated. Many died due to stenosis of the gastrointestinal tract or infection. Thirteen months after irradiation 15 males and 13 females survived (Table I). Thirteen of the 15 males had tumors and all were gastric tumors. The histological type of gastric tumors was well differentiated and/or poorly differentiated. They were located independently (Figs. 1 and 2). Signet ring cells that appeared in poorly differentiated carcinomatous tissues were mostly stained with periodic acid-Schiff (PAS) or Alcian blue by PAS-Alcian blue and were Alcian blue-positive in high iron diamine-Alcian blue staining. Three of 13 tumors were signet ring cell carcinomas (vs. non-irradiated, $P<0.01$), one poorly differentiated type and 13 well differentiated types (vs. non-irradiated, $P<0.01$). In females, 13 animals had tumors, and 9 out of 13 were well differentiated gastric tumors (vs. non-irradiated, $P<0.01$), 2 had signet ring cell carcinomas (vs. non-irradiated, $P<0.05$) and 1 had poorly differentiated adenocarcinomas. Of the total of 5 signet ring carcinomas observed in both sexes, four had invaded the regional lymph nodes but had not metastasized to distant organs (Figs. 3 and 4). Liver tumors were also observed. Some animals had two or three tumors, well differentiated,

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[2] Abbreviations: MNNG, N-methyl-N′-nitro-N-nitrosoguanidine; ENNG, N-ethyl-N′-nitro-N-nitrosoguanidine; NBU, N-nitrobutylurea; PAS, periodic acid-Schiff; C_s^b, hypocatalasemic mice.

Table I. Incidence of Tumor in X-Irradiated C_s^b Mice

Sex	No. of mice at start	No. of mice		Gastric tumor			Liver tumor
		Effective	Tumor bearing[a] (%)	Poorly differentiated type	Signet ring cell carcinoma	Well differentiated type	
X-irradiated animals							
M	32	15	13** (87)	1 (7%)	3** (20%)	13** (87%)	4 (27%)
F	33	13	11** (85)	1 (8%)	2* (15%)	9** (69%)	2 (15%)
Total	65	28	24** (86)	2 (7%)	5** (18%)	22** (79%)	6 (21%)
Non-irradiated animals							
M	60	55	20[b] (36)	0	0	0	19 (35%)
F	65	62	18[c] (29)	0	0	0	5 (8%)
Total	125	117	38 (32)	0	0	0	24 (21%)

a) Included poorly differentiated, well differentiated gastric tumors and/or liver tumors.
b) Other tumor: harderian gland.
c) Other tumors; ovary, mammary.
** Significantly different from non-irradiated animals ($P<0.01$).
* Significantly different from non-irradiated animals ($P<0.05$).

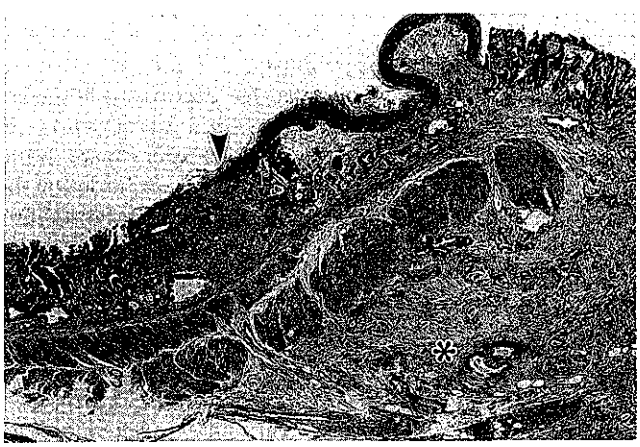

Fig. 1. Foci of signet ring cell carcinoma in mucosa (▼) and well differentiated adenocarcinoma (✻) invading into subserosal muscle layers.

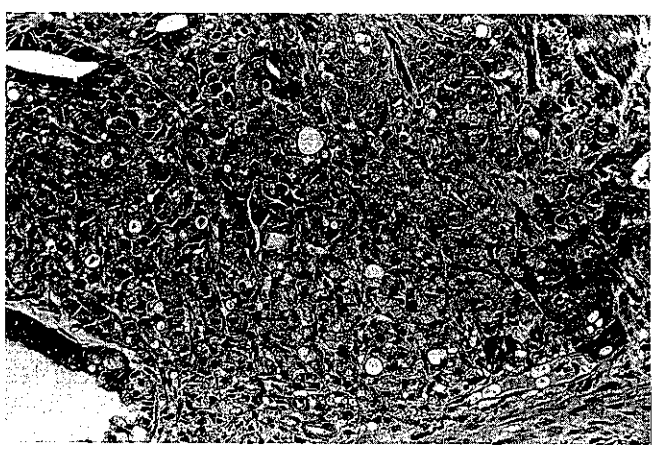

Fig. 2. Higher magnification of foci of signet ring cell carcinoma in Fig. 1.

poorly differentiated gastric tumors and/or liver tumors. In non-irradiated animals, liver tumors appeared in 34.5% of male mice during the 13-month observation period, and in the females 18% were ovary tumors, but there were no gastric tumors of well or poorly differentiated type in the glandular stomach. In C3H mice, the study is being continued.

It can be concluded that different strains have different susceptibility to the induction of various types of gastric carcinomas by radiation. The origin of signet ring cell carcinoma is a matter of debate; it is not clear whether it arises from well differentiated adenocarcinoma or whether it occurs *de novo*. In the present study, foci of signet ring cell carcinoma and well differentiated adenocarcinoma appeared independently, as reported in other studies.[1-3] Thus, the origin of signet ring cell carcinoma seems to be independent of that of well differentiated adenocarcinoma.

In summary, we have described the occurrence of signet ring cell carcinomas in the glandular stomach after localized X-irradiation in C_s^b.

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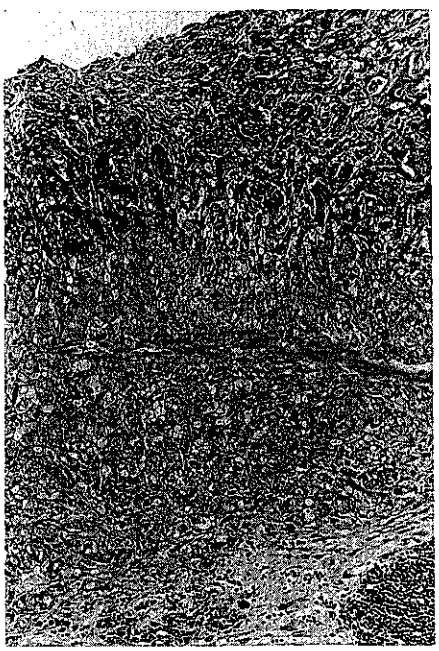

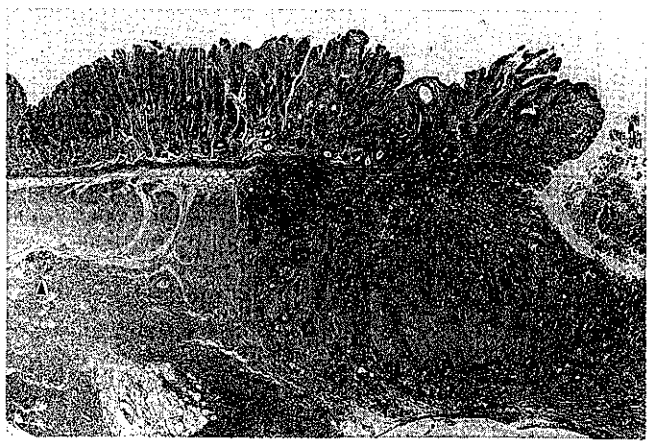

Fig. 4. Mass of neoplastic cell foci invading serosa and lymph node (▲). (Alcian blue-PAS staining)

Fig. 3. Change of poorly differentiated carcinoma to signet ring cell carcinoma in mucosa and invasion into muscle layer.

REFERENCES

1) Fujita, M., Takami, M., Ushigane, M., Nampei, S. and Taguchi, T. Enhancement of gastric carcinogenesis in dogs given N-methyl-N′-nitro-N-nitrosoguanidine following vagotomy. *Cancer Res.*, **39**, 811–816 (1979).
2) Sunagawa, M., Takeshita, K., Nakajima, A., Ochi, K., Habu, H. and Endo, M. Duration of ENNG administration and its effect on histological differentiation of experimental gastric cancer. *Br. J. Cancer*, **52**, 771–779 (1985).
3) Kurihara, M., Yamada, F., Maruyama, T., Miyasaki, K., Izumi, T., Tabata, M., Kinoshita, Y. and Shirakabe, H. Effect of gastrin on the production of gastric carcinoma in dogs induced by ENNG. *Igaku-no-Ayumi*, **104**, 663–665 (1978) (in Japanese).
4) Watanabe, H., Hirose, F., Takizawa, S., Terada, Y., Fujii, I. and Ohkita, T. A mode of incipient growth in chemically induced signet ring cell carcinoma of the canine stomach. *Pathol. Res. Pract.*, **164**, 216–223 (1979).
5) Tatematsu, M., Takahashi, M., Fukushima, S., Hananouchi, M. and Shirai, T. Effects in rats of sodium chloride on experimental gastric cancers induced by N-methyl-N′-nitro-N-nitrosoguanidine or 4-nitroquinoline-1-oxide. *J. Natl. Cancer Inst.*, **55**, 101–106 (1975).
6) Tahara, E. and Haizuka, S. Effect of gastro-entero-pancreatic endocrine hormones on the histogenesis of gastric cancer in rats induced by N-methy-N′-nitrol-N-nitorosoguanidine; with special reference to development of scirrhous gastric cancer. *Gann*, **66**, 421–426 (1975).
7) Kojima, A. and Watanabe, H. Mouse model of stomach tumor, initiated by X-irradiation and promoted by chronic gastritis. *Proc. Jpn. Cancer Assoc., 43rd Annu. Meet.*, 58 (1984).
8) Hirose, F. Experimental induction of carcinoma in the glandular stomach by localized X-irradiation of gastric region. *Gann Monogr.*, **8**, 75–113 (1969).
9) Watanabe, H., Naito, M., Kawashima, K. and Ito, A. The effects of pretreatment with stomach extract on the incidence of X-ray-induced gastric tumor in ICR mice. *Gann*, **75**, 884–888 (1984).
10) Feinstein, R. N., Howard, J. B., Braun, J. T. and Seaholm, J. E. Acatalasemic and hypocatalasemic mouse mutant. *Genetics*, **53**, 923–933 (1966).
11) Ito, A., Watanabe, H., Naito, M., Naito, Y. and Kawashima, K. Correlation between induction of duodenal tumor by hydrogen peroxide and catalase activity in mice. *Gann*, **75**, 17–21 (1984).
12) Watanabe, H., Aoyama, H., Nakagawa, Y., Mori, M., Ito, A., Sawada, S. and Yokoro, K. Liver tumorigenesis induced by the fast neutron in hypocatalasemic mice (C3H/C$^{b}_{s}$. *Nagasaki Igaku Zasshi*, **61**, 391–323 (1986) (in Japanese).