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Increased Susceptibility to N-Nitrosomethylurea Gastric Carcinogenesis in Transforming Growth Factor α Transgenic Mice with Gastric Hyperplasia

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Glandular stomach carcinogenesis after N-nitrosomethylurea (NMU) treatment was examined in transgenic mice bearing a human transforming growth factor alpha (TGF-α) cDNA driven by the mouse metallothionein-I promoter (mouse line MT100) in the inbred mouse line FVB/N. Untreated MT100 mice exhibit a severe age-related gastric fundic hyperplasia. Both sexes of MT100 mice were given 10 weekly intragastric intubations of 0.5 mg NMU per mouse from 6 weeks of age and/or zinc chloride in drinking water to stimulate transgene expression from 5.5 weeks of age to the experiment termination. Animals were killed sequentially at 10, 19 and 29 experimental weeks. Several histochemical markers (AB-PAS, TGF-α, pepsinogen isozyme 1, proliferating cell nuclear antigen) were used. Abnormal histochemical patterns were found in untreated MT100 and NMU-treated MT100 mice for all 4 markers of differentiation and carcinogenesis. Precancerous lesions including atypical and/or adenomatous hyperplasia were found in the fundic region of 16/22 male and 8/22 female MT100 mice but not in 27 male and 24 female FVB/N mice treated with NMU. One of 22 MT100 males had fundic carcinoma. FVB/N mice treated with NMU had neither precancerous lesions nor carcinomas in the fundus. Well differentiated adenocarcinomas in the pyloric region were induced at incidences of 2/22 male and 1/22 female MT100 mice treated with NMU and 4/27 male and 4/24 female FVB/N mice treated with NMU. Both strains also had a high incidence (55 to 92%) of squamous cell carcinomas of the forestomach. In conclusion, TGF-α induced a hyperplastic lesion in the gastric fundus that appeared to predispose the MT100 mice to carcinogenesis by NMU.

Key words: N-Nitrosomethylurea — Glandular stomach carcinoma — Transgenic mouse — Transforming growth factor α

The role of TGF-α[6] is being elucidated *in vivo* by introducing into mice a recombinant TGF-α transgene whose expression is regulated by a variety of tissue-specific promoters.[1,2] TGF-α participates in the development of a variety of spontaneous cancers[3–6] while collaborating with other factors including c-*myc*.[7–10] TGF-α also accelerates the development of carcinogen-induced cancer.[11–13] We have established and maintained a transgenic line bearing a human TGF-α cDNA driven by the mouse metallothionein-I promoter (mouse line MT100) in the inbred mouse line FVB/N.[3,14] These transgenic mice are characterized by extraordinary structural and functional changes in the glandular stomach.[14,15] Structurally, the mice develop severe cystic hyperplasia containing mucous-laden secretions in the fundic gastric mucosa, but not in the pyloric region. Foci of dysplastic cells were seen in the lesions of mice surviving until later stages of life. The functions of parietal and chief cells were reduced with a decrease in the expression of genes encoding H^+, K^+-ATPase and pepsinogen C.

Phenotypic expression of Pg1 has been demonstrated in rat pyloric glands.[16,17] In chemical carcinogenesis of the rat glandular stomach, the Pg1-deficient alteration is detected not only in the gastric tumors but also in the histologically normal pyloric glands.[18,19] This altered pepsinogen isozyme pattern termed PAPG was considered a preneoplastic lesion in the glandular stomach of rats.[20,21] It is also confirmed that Pg1 immunoreactivity was present in the mucous neck and chief cells of mouse stomach using rabbit anti-rat Pg1 serum.[22]

Tatematsu *et al.* have shown that the NMU-induced glandular stomach cancer model in the mouse exhibits

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[6] Abbreviations used are: TGF-α, transforming growth factor α; NMU, N-nitrosomethylurea; Pg1, pepsinogen isozyme 1; PAPG, Pg1-altered pyloric glands; PCNA, proliferating cell nuclear antigen; AB-PAS, alcian blue-periodic acid Schiff; HE, hematoxylin and eosin; SCC, squamous cell carcinoma.

similarities to human gastric cancers.[23, 24] In contrast, mice are resistant to N-methyl-N′-nitro-N-nitrosoguanidine stomach carcinogenesis.[25]

In this study, we assessed the susceptibility of the MT100 transgenic mouse stomach to NMU, and whether Pg1 was modified in NMU-initiated gastric carcinogenesis of the glandular stomach.

MATERIALS AND METHODS

Animals and chemicals Mice were obtained from mating of male transgenic heterozygous mice (+/−) harboring the metallothionein-human TGF-α fusion gene (mouse line MT100) to female negative FVB/N (−/−) (Harlan Sprague Dawley, Inc., Indianapolis, IN). MT100 transgenic mice were identified as transgenic at necropsy by the presence of a grossly rigid and thickened pancreas.[13] Mice were maintained in accordance with procedures in the NIH Guide, and supplied with feed (Purina 5010 autoclavable rodent meal, Ralston Purina Co., St. Louis, MO) and tap water *ad libitum*. Mice were weaned at 4 weeks of age. The animals were observed daily for abnormalities, and body weights were recorded weekly.

NMU was purchased from Sigma Chemical Co. and stored in a freezer. NMU was dissolved in saline and acidified with 3% acetic acid to pH 4.0. The NMU solution was made up fresh each time before use.

Experimental design and necropsy Ninety-nine males and 93 females were randomly divided into 4 groups of each sex with approximately the same numbers after weaning. Mice from groups 2 and 4 were administered 50 m*M* zinc chloride (acidified with hydrochloric acid to pH 2.5) in drinking water to stimulate transgene expression from 5.5 weeks of age for 2.5 weeks, and then 25 m*M* zinc chloride from 8 weeks of age to the experiment termination. Mice from groups 3 and 4 were gavaged with 0.5 mg of NMU per mouse once a week for a total of 10 weeks from 6 weeks of age. Mice from groups 1 and 2 were given saline gavage in the same manner as mice in groups 3 and 4. Animals were killed by carbon dioxide inhalation and necropsied at experimental weeks 10 (16 weeks old), 19 and 29 after the commencement of NMU gavage. Dead and moribund animals were also necropsied.

Histopathological and immunohistochemical procedures The stomachs were inflated with 10% neutral buffered formalin for 5 min, placed and trimmed into 6 strips, fixed in formalin and embedded in paraffin. Two stomachs from each group were placed in Bouin's fixative for subsequent TGF-α and Pg1 immunohistochemistry at experimental weeks 19 and 29. Other gross lesions were recorded and also fixed in 10% neutral buffered formalin and embedded in paraffin. Tissue sections were stained with HE and AB-PAS. Stomach preneoplastic or neoplastic lesions were classified according to criteria defined elsewhere.[23-25]

Immunohistochemical staining for TGF-α was performed by the methods described.[26] Pg1 immunohistochemical staining was generously done by Dr. M. Tatematsu (Aichi Cancer Center Research Institute, Nagoya)[21] using a rabbit anti-rat pepsinogen I serum (at 1:15,000 dilution), which was generously provided by Dr. C. Furihata, Department of Molecular Oncology, Institute of Medical Science, University of Tokyo.[17] PCNA immunohistochemical staining was performed as previously reported.[13] Briefly, after deparaffinization, endogenous peroxidase in tissue sections was quenched in 2% hydrogen peroxide for 15 min. Slides were washed and then placed in water in a plastic jar covered with a loose-fitting plate. The slides were twice heated in a microwave oven for 5 min. After cooling at room temperature, slides were rinsed in phosphate-buffered saline. Sections were stained for PCNA by the avidin-biotin peroxidase complex (ABC) technique, using a mouse monoclonal antibody to PCNA (at 1:400 dilution) (DAKO Corp., Carpinteria, CA) and a mouse Vectastain Elite ABC kit (Vector Laboratories, Burlingame, CA).

RNA analysis Zinc chloride (5 mg/kg) was injected intraperitoneally into MT100 mice, 5 or 43 weeks of age. Mice were killed and their stomachs removed for RNA isolation at 6 or 10 h post-injection. Total stomach RNA was isolated and analyzed using Northern blot hybridization as described elsewhere.[3, 14] Fifteen μg of total RNA was loaded per lane, and hybridization was performed using a ^{32}P-radiolabeled 925 bp human TGF-α cDNA probe.[3]

Statistical analysis Data on cumulative mortality were analyzed by means of generalized Kruskal/Wallis analysis.[27] The incidence of stomach lesions and tumors were analyzed by using the one-tailed Fisher's exact probability test.

RESULTS

Mortality The first death from forestomach tumors occurred at week 9 for a male MT100 transgenic mouse treated with NMU. Therefore, effective numbers of animals were determined based on the mice alive after week 9. The numbers of deaths and moribund cases are presented in Tables I and II. Mortalities were not significantly different in each corresponding treatment between MT100 and FVB/N mice, while the mortalities of male FVB/N NMU mice and female FVB/N NMU+zinc chloride mice were significantly higher than those of male FVB/N control and zinc chloride mice, and female FVB/N zinc chloride mice, respectively. Almost all moribund mice and deaths in mice treated with NMU were associated with the presence of invasive malignant tumors in the forestomach.

Table I. Tumor Incidences of Glandular Stomach and Forestomach in Male MT100 and FVB/N Mice from 9 to 29 Weeks

Strain	Treatment	Total No. of mice examined[a]	Glandular stomach[b]					Forestomach[b]	
			Fundic region			Pyloric region			
			Atypical hyperplasia	Adenomatous hyperplasia	Carcinoma	Adenomatous hyperplasia	Carcinoma	Papilloma	Squamous cell carcinoma
MT100	Control	10 (1)	4 (40)	1 (10)	0	0	0	0	0
	$ZnCl_2$	8 (1)	2 (25)	0	0	0	0	0	0
	NMU	11 (4)	8 (73)[d]	4 (36)[c]	1 (9)	5 (45)	1[g] (9)	3 (27)	6 (55)
	NMU+$ZnCl_2$	11 (3)	8 (73)[d]	1 (9)	0	0	1[f] (9)	1 (9)	6 (55)[c]
FVB/N	Control	13 (0)	0	0	0	0	0	0	0
	$ZnCl_2$	14 (0)	0	0	0	0	0	0	0
	NMU	14 (9[e])	0	0	0	2 (14)	1[f] (7)	3 (21)	11 (79)
	NMU+$ZnCl_2$	13 (2)	0	0	0	2 (15)	3[f,h] (23)	2 (15)	12 (92)

a) Dead or moribund cases in parentheses.
b) Percentages in parentheses.
c), d) Significantly different from FVB/N NMU or NMU+$ZnCl_2$ mice at $P<0.05$, 0.001, respectively.
e) Significantly different from FVB/N control and zinc chloride mice at $P<0.001$.
f) Well differentiated carcinoma.
g) Poorly differentiated carcinoma.
h) Associated with one signet ring cell carcinoma.

Table II. Tumor Incidences of Glandular Stomach and Forestomach in Female MT100 and FVB/N Mice from 9 to 29 Weeks

Strain	Treatment	Total No. of mice examined[a]	Glandular stomach[b]					Forestomach[b]	
			Fundic region			Pyloric region			
			Atypical hyperplasia	Adenomatous hyperplasia	Carcinoma	Adenomatous hyperplasia	Carcinoma	Papilloma	Squamous cell carcinoma
MT100	Control	10 (1)	0	0	0	0	0	0	0
	$ZnCl_2$	9 (2)	0	0	0	0	0	0	0
	NMU	13 (4)	6 (46)[c,e]	2 (15)	0	2 (15)	1[g] (8)	4 (31)	6 (46)[d]
	NMU+$ZnCl_2$	9 (1)	2 (22)	1 (11)	0	0	0	1 (11)	5 (56)
FVB/N	Control	13 (2)	0	0	0	0	0	0	0
	$ZnCl_2$	8 (0)	0	0	0	0	0	0	0
	NMU	12 (5)	0	0	0	3 (25)	2[g,h] (17)	2 (17)	11 (92)
	NMU+$ZnCl_2$	12 (6[f])	0	0	0	0	2[g] (17)	4 (33)	8 (67)

a) Dead or moribund cases in parentheses.
b) Percentages in parentheses.
c), d) Significantly different from FVB/N NMU or NMU+$ZnCl_2$ mice at $P<0.05$, 0.001, respectively.
e) Significantly different from MT100 control or $ZnCl_2$ mice at $P<0.05$.
f) Significantly different from FVB/N zinc chloride mice at $P<0.05$.
g) Well differentiated carcinoma.
h) Associated with one signet ring cell carcinoma.

Macroscopic findings The fundic mucosal surface of the NMU-untreated and -treated glandular stomach in MT100 mice was irregular and thickened (Fig. 1). Several small white nodules were observed in the pyloric mucosa of both NMU-treated MT100 and FVB/N mice. White/gray nodules and tumor masses were found in the forestomach of both strains of mice treated with NMU.

Histopathological glandular stomach findings 1. Fundic region A summary of the incidence of glandular stomach lesions is shown in Tables I and II. Statistical comparison of tumor incidences between MT100 and FVB/N was available, since mortalities were not statistically significantly different in each corresponding treatment between MT100 and FVB/N mice. From week 10, the epithelium generally revealed diffuse cystic hyperplasia associated with inflammatory cells and fibrosis in the lamina propria of the fundic region in both sexes of the NMU-treated and -untreated MT100 transgenic mice (Fig. 2). This lesion became more severe (measured by thickness of epithelium and increased numbers and size of cystic

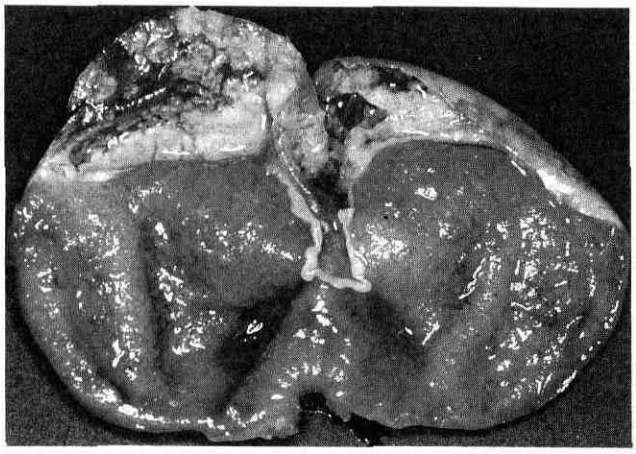
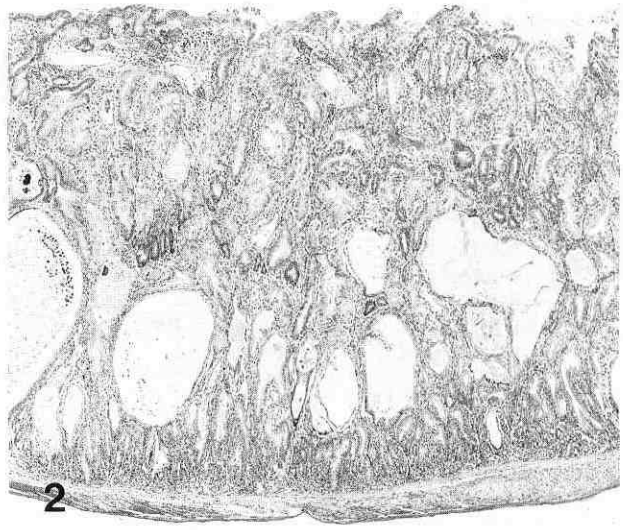


Fig. 1. Macroscopic appearance in a male MT100 control mouse, 25 weeks of age. Note thickening and irregular mucosa in the fundic region. FVB mice have no thickenings.



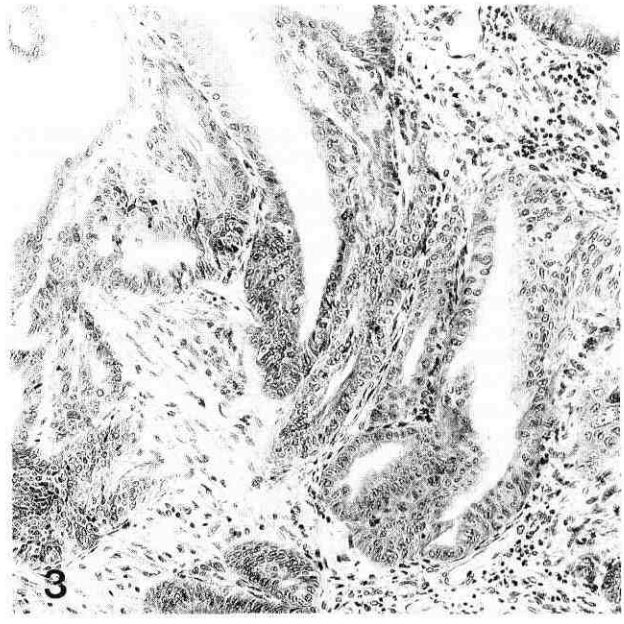


Fig. 2. Cystic hyperplasia in a male MT100 control mouse, 25 weeks of age. HE, ×30.

Fig. 3. Mucosal atypical hyperplasia in the fundic region in a male MT100 control mouse. HE, ×150.

glands) with age. No cystic hyperplasias were found in the pyloric region of the MT100 mouse stomach. Focal mucosal atypical hyperplasia was characterized by an intraepithelial lesion of proliferating epithelial cells with atypical hyperchromatic nuclei, scant cytoplasm and loss of cellular polarity (Fig. 3). Until week 10, atypical hyperplasias were found in 2/4 (50%) and 3/4 (75%) male MT100 NMU and NMU+zinc mice, and in 2/4 (50%) female MT100 NMU mice. This lesion was induced at higher incidences (67 to 100%) in male MT100 NMU and NMU+zinc mice than in females (33 to 50%) until weeks 19 and 29. Atypical hyperplasias were also observed in male MT100 control (50%) and/or zinc (67%) mice but not in females until weeks 19 and 29. Adenomatous hyperplasia was characterized by an exophytic epithelial cell proliferation (sometimes polypoid), marked hyperchromatic nuclei, slight cellular atypia and less cytoplasm than in adjacent epithelial cells. A few lesions were endophytic. Adenomatous hyperplasias were found in 2/4 (50%) and 1/7 (14%) male MT100 NMU and NMU+zinc mice, and in 1/5 (20%) female MT100 NMU+zinc mice until week 19 (Fig. 4). Only 1 male MT100 control (25%) had this lesion. The yields of this lesion were age-related with 2/3 (67%) and 2/6 (33%) male and female MT100 NMU mice, with no significant inter-group differences at week 29. Well-differentiated adenocarcinoma in the fundic region was found in 1/3 (33%) male MT100 NMU mice at week 29 (Fig. 5). In contrast, no preneoplastic and neoplastic lesions in the fundic region of FVB/N mice were evident in any of the groups.

2. Pyloric region The untreated MT100 and FVB/N mice had no lesions. Atypical hyperplasia developed in 1/4 (25%) male MT100 NMU+zinc mice until week 10, while it was found in 1/6 (17%) female MT100 NMU mice only until week 29. Adenomatous hyperplasias and well differentiated adenocarcinoma were observed in 3/4 (75%) male MT100 NMU and 1/7 (14%) male MT100 NMU+zinc mice until week 19, respectively. At week 29, adenomatous hyperplasias and poorly differentiated adenocarcinoma were found in 2/3 (67%) and

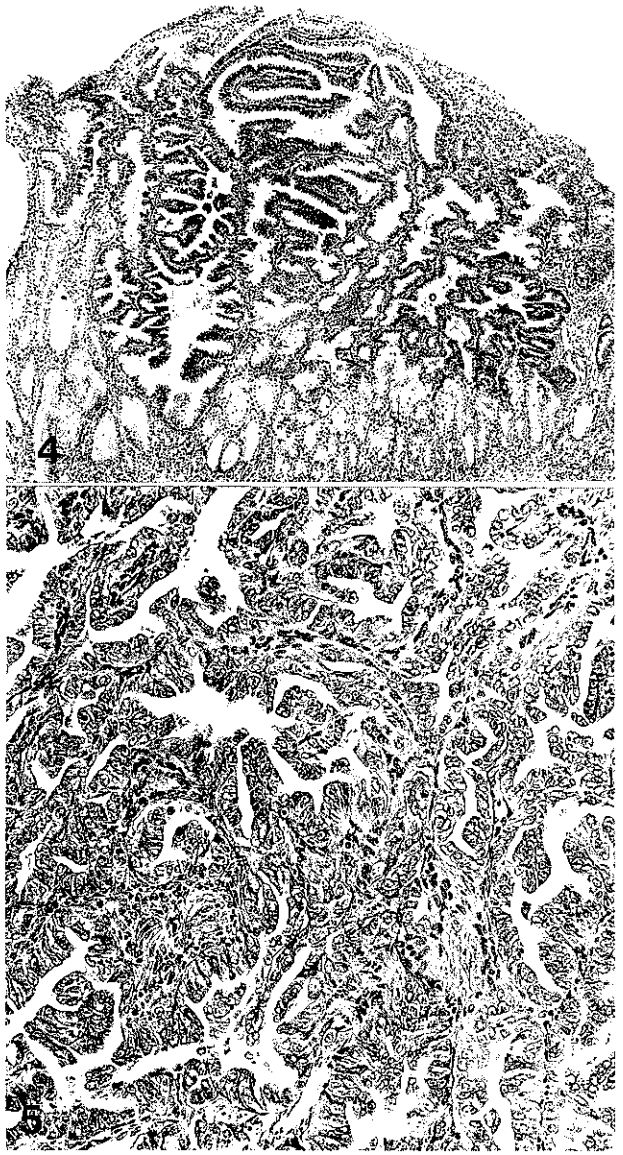


Fig. 4. Adenomatous hyperplasia in the fundic region of a male MT100 NMU mouse. HE, ×50.

Fig. 5. Well-differentiated adenocarcinoma in the fundic region of a male MT100 NMU mouse. HE, ×150.

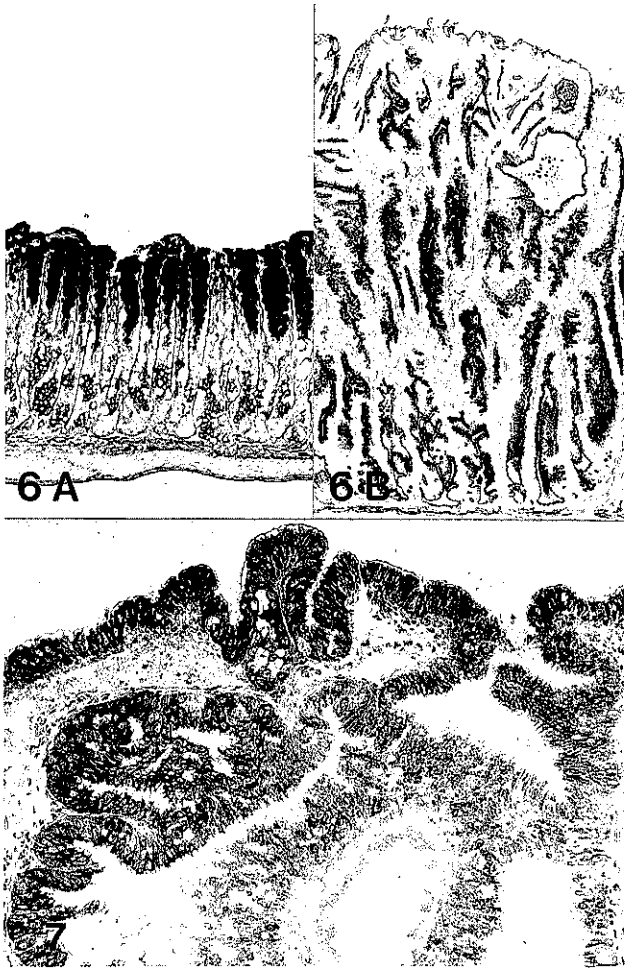


Fig. 6. (A) Normal fundic region in a male FVB/N NMU mouse. AB-PAS, ×100. (B) Cystic hyperplasia in the fundic region in a male MT100 $ZnCl_2$ mouse. AB-PAS, ×30.

Fig. 7. TGF-α was expressed diffusely in adenomatous hyperplasia of the fundic region in a male MT100 mouse. ×150.

1/3 (33%) male MT100 NMU mice. Adenomatous hyperplasias and well differentiated adenocarcinoma were seen in 2/6 (33%) and 1/6 (17%) female MT100 NMU mice at week 29. Mucosal atypical hyperplasias were also found in both sexes of FVB/N NMU and NMU+zinc mice until week 19. Adenomatous hyperplasias, well differentiated adenocarcinomas and signet ring cell carcinomas were found in 2/3 (25–43%), 1/3 (13–43%) and 1/7 (14%) of both sexes of FVB/N NMU and/or NMU+zinc mice until week 29, respectively.

3. AB-PAS histochemistry The foveolar epithelium stained red-purple. AB-PAS was limited to the foveolar epithelium of FVB/N stomachs (Fig. 6A). In contrast, in the fundic region of untreated and NMU MT100 mice, AB-PAS positive staining was seen in cystic hyperplasia in the cytoplasm of the epithelium (Fig. 6B). The cytoplasm of foveolar epithelium in the pyloric region of MT100 transgenic and FVB/N mice was AB-PAS positive. The epithelium of adenomatous hyperplasias and adenocarcinomas was negative with AB-PAS staining.

Immunohistochemistry of the glandular stomach 1. TGF-α In untreated MT100 stomach, TGFα was over-

Fig. 8. (A) Expression of Pg1 in normal pyloric gland in a male MT100 control mouse. ×150. (B) Pg1 expression was greatly reduced in atypical hyperplasia in the pyloric region of MT100 mouse given NMU. ×150.

Fig. 9. (A) Normal fundic region in a female FVB/N control mouse. Pg1, ×150. (B) Cystic hyperplasia in the fundic region in a male MT100 control mouse. Pg1, ×150.

expressed predominantly in the fundus, and considerably less in the pylorus and forestomach.[15] TGF-α was also focally expressed in atypical hyperplasia, adenomatous hyperplasia and carcinoma on the epithelial cell membrane and/or cytoplasm of epithelium in the fundic region (Fig. 7). No TGF-α overexpression was seen in

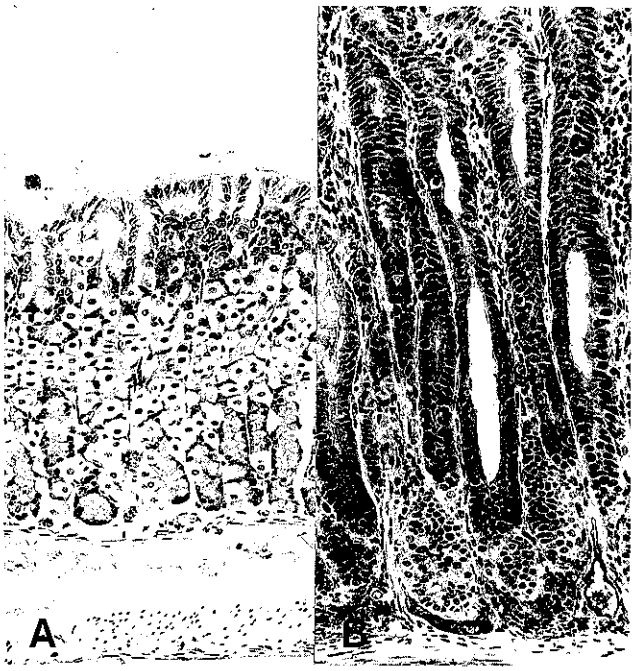


Fig. 10. (A) Normal fundic region in a male FVB/N $ZnCl_2$ mouse. PCNA, ×150. (B) Cystic hyperplasia in the fundic region in a male MT100 control mouse. PCNA, ×150.

cystic hyperplasias or in carcinomas in the pyloric region. In FVB mice, TGF-α expression was not seen in normal epithelium or in any lesion in any portion of the stomach.

2. Pg1 Pg1 immunohistochemical staining was seen specifically in the mucous neck cells and chief cells in the pyloric glands and diffusely in the normal mouse glandular stomach using the rat Pg1 primary antibody (Fig. 8A). In both MT100 and FVB/N mice, atypical hyperplasias and tumors in the pyloric region showed weak or negative Pg1 expression (Fig. 8B). In FVB/N mice, Pg1 was diffusely expressed in mucous neck cells and chief cells (Fig. 9A). In contrast, in MT100 mice, Pg1 was expressed in the fundic region in the cytoplasm of mucous neck cells and chief cells, in which Pg1 expression was focally deficient (Fig. 9B). Also, Pg1-immunoreactive epithelial cells did not appear morphologically as typical normal chief cells.

3. PCNA PCNA immunohistochemical staining was performed in representative sections of male MT100 and FVB/N control and zinc chloride-treated mice. The nuclei of epithelial cells in the isthmus and neck zones were PCNA immunoreactive in both fundic and pyloric regions of FVB/N mice (Fig. 10A), and the pyloric region of MT100 mice. In contrast, in the fundic regions of MT100 mice, PCNA immunoreactivity was found in the isthmus and neck zone and also in the irregular

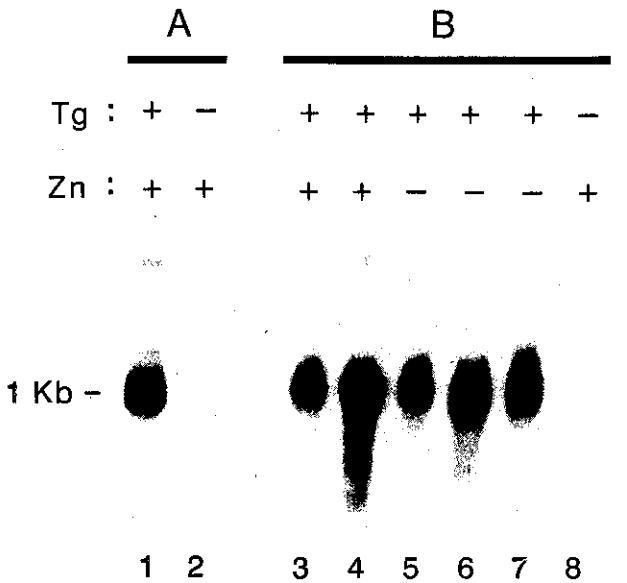


Fig. 11. Effect of exposure to zinc on expression of the TGF-α transgene in the stomach of MT100 mice. Zinc chloride was injected intraperitoneally into mice of (A) 10 months or (B) 5 weeks of age. Mice were killed and their stomachs removed for RNA isolation either 6 h (lanes 1–3) or 10 h (lanes 4–8) post-injection. Information at the top indicates (Tg) whether the mouse was transgenic (+ is MT100) or not (− is FVB/N), and (Zn) if the mouse received zinc or not (+ or −, respectively). Fifteen μg of total gastric RNA was loaded on an agarose gel per lane. After electrophoresis and transfer, the blot was hybridized to a human TGF-α probe, as described in "Materials and Methods." The resulting autoradiogram is presented.

proliferating zone (Fig. 10B). In MT100 mice, it was more extensive than in FVB mice.

Tumors in other tissues A summary of tumor incidences in the forestomach is given in Tables I and II. SCCs were found at high incidences (46 to 92%) in both strains of mice treated with NMU. Interestingly, tumor incidences in male and female FVB/N NMU mice were significantly higher than those of MT100 mice. These findings may be related to the lower survival of MT100 mice since 2/3 male and 4/6 female MT100 mice receiving NMU and surviving to week 29 had SCC. The higher incidence of SCCs produced higher mortality in male and female FVB/N NMU mice, since tumors invaded the serosa and metastasized to the peritoneum. A high incidence of hyperplastic and precancerous squamous lesions (data not shown) including papillomas was also seen. Lung adenomas (17 to 33%) and adenocarcinomas (7 to 31%) were observed in both NMU- and NMU + zinc chloride-treated FVB/N male and female mice. In contrast, a lung adenoma was found in only one male MT100 mouse given NMU. Duodenal adenocarcinomas were seen in one female MT100 and one female FVB/N NMU mouse (data not shown).

Transgene expression in stomach Human TGF-α RNA transcripts were highly abundant in the stomachs of MT100 mice, as judged by Northern blot hybridization (Fig. 11).[14] As expected, transgenic RNA was not detected in the FVB/N mouse stomach. Interestingly, levels of human TGF-α RNA were not overly increased in the stomach after intraperitoneal injection of zinc chloride, indicating that zinc could not effectively stimulate transgene expression in this organ.

DISCUSSION

Our data reveal that intragastric intubation of NMU induces precancerous lesions and gastric carcinoma in the fundic region only of MT100 mice, while NMU also induced carcinoma in the pyloric region of both MT100 mice and FVB/N mice. This is the first report describing the induction of gastric carcinoma in the mouse fundic region by NMU, implicating TGF-α as a cocarcinogen in this tissue. MT100-specific lesions are restricted to the fundus because the TGF-α transgene is most active in the MT100 fundus.[15] The MT100 mice exhibit age-related fundic diffuse cystic hyperplasia and loss of normal differentiation[14, 15, 28] which increases susceptibility to fundic carcinoma and its precancerous lesions. Moreover, a higher incidence of carcinoma might have been seen if the mice had lived longer. In the present studies, NMU enhanced the appearance of atypical hyperplasias found within cystic hyperplasias, already evident in untreated MT100 males. Atypical hyperplasia in aging MT100 mice appears to represent a precursor of adenomatous hyperplasia and/or adenocarcinoma in NMU-treated MT100 mice. These lesions were never seen in the fundic region of NMU-treated FVB/N mice. Meanwhile, the earlier deaths in FVB/N mice resulted from development of SCCs in NMU-treated mice. A similar high incidence of SCCs was previously demonstrated in other mouse strains given NMU intragastrically.[23] However, a dramatic decrease of SCC development in the forestomach has been seen when NMU is given in the drinking water.[24]

Gastric tumors have been reported in several transgenic mouse lines. Adenovirus types 12 E1A and E1B transgenic mice developed adenocarcinoma or adenosquamous carcinoma at or near the squamocolumnar junction.[29] Rous sarcoma virus long terminal repeat-Shope growth factor transgenic mice developed severe epithelial atypias and exophytic neoplasias.[30] The majority of human gastric carcinomas, however, arise in the pyloric region. NMU yielded gastric carcinomas in the pyloric region of MT100 and FVB/N mice with similar

incidences. In the pyloric region of MT100 control mice, there was no epithelial hyperplasia.

Pg1 expression was specifically demonstrated in the pyloric glands and basal zone of the fundic region of transgenic and control mouse glandular stomach using anti-rat Pg1 primary antibody. The presence of PAPG, preneoplastic lesions in the glandular stomach of rats, was recognized in pyloric glands of both MT100 and FVB/N mice treated with NMU. Pg1 expression was weak or negative in the cytoplasm of the cells in atypical hyperplasias and tumors in the pyloric region.

In the fundic region of MT100 mice stomach, AB-PAS staining suggested that the proliferating epithelium originated from the pit cells. Furthermore, these proliferating cells were immunoreactive for PCNA, as were the cells in the isthmus zone. In contrast, chief cells declined in numbers.[15, 28] In fact, Pg1 expression showed lower levels in basal cells of MT100 transgenic mice than in those of FVB/N mice. Therefore, chief cells might also have decreased cell functions in MT100 mice, or cells with Pg1 expression in the fundus may be undifferentiated chief cells.[15]

It has been suggested that TGF-α participates in the development of gastric carcinomas. In adult human gastric mucosa, TGF-α was strongly expressed in gastric cancer cells as well as in the foveolar epithelium with regenerative or hyperplastic change.[31, 32] TGF-α appeared to be produced by gastric tumor cells in an autocrine manner.[31] In this study, TGF-α determined by immunohistochemistry was specifically expressed in the epithelial membranes of atypical hyperplasias, adenomatous hyperplasias and adenocarcinoma in the NMU-treated transgenic fundus. Therefore, this result strongly suggests a role of TGF-α in gastric carcinogenesis.

It was noted in the present study that exposure to zinc did not overly influence the incidence of proliferative gastric lesions in MT100 mice. This finding is most likely due to the fact that in MT100 mice, zinc did not stimulate transgenic expression more than 5-fold in most tissues[3] and had almost no effect on transgene expression in the stomach where expression was constitutively high.[14] Our present study with zinc and NMU confirms these previous findings.

In conclusion, NMU may have induced gastric carcinoma in the fundic region of the MT100 mouse glandular stomach by enhancing conversion of atypical hyperplasia into adenomatous hyperplasia or adenocarcinoma within a diffusely cystic hyperplastic epithelium.

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