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Histopathological and experimental studies on development of gastric cancers

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ABSTRACT

A mode of development of diffuse type cancers and adenocarcinomas was studied. Materials were small cancers endoscopically diagnosed, and minute cancers incidentally found in the gastric mucosa of resected human stomachs. In order to analyze initial lesions of signet ring cell carcinomas, we induced experimentally signet ring cell cancers in dog stomachs by administration of N-ethyl-N'-nitro-N-nitrosoguanidine (ENNG). From these studies, it was shown that both signet ring cell carcinomas and adenocarcinomas arise at the neck region of gastric glands. Signet ring cell carcinomas arise de novo from the stem cell of normal-looking glandular tubules. There were no precursor lesions for the development of this type cancer. Adenocarcinomas seemed to arise in the incompletely intestinalized glandular tubules or arise de novo at the neck region of pyloric glandular tubules. From mucin-immunohistochemical studies, it was found that about 23% of adenocarcinomas were gastric-type, 30% were intestinal-type and the remaining 47% were mixed of gastric and intestinal phenotype.

INTRODUCTION

From a histogenetic point of view, gastric cancers are divided into two main histological types; diffuse and intestinal type. The former is thought to arise in the proper gastric mucosa, which is not involved in atrophic change or in chronic gastritis. However, a mode of the development of diffuse type cancers has not been fully elucidated. On the other hand, most intestinal type cancers comprise adenocarcinomas, which are thought to arise in the intestinalized gastric mucosa. But, a relationship between intestinal metaplasia and adenocarcinomas development is not fully defined. This study was undertaken to find out how both types of gastric cancers arise in the gastric mucosa. A special attention has been focused on initial lesions of signet ring cell carcinomas and adenocarcinomas. In order to study cell proliferation of the cancer tissues and the background gastric mucosa, ^{3}H-thymidine autoradiography, and BrdU(bromodeoxyuridine)/PCNA(proliferating cell nuclear antigen) immunohistochemistry were used. Mucin phenotypes of gastric cancers were assessed by immunohistochemistry using MUC2, MUC5AC, MUC6 and CD10 monoclonal antibody. Amplifications of several oncogenes and expression of oncogene products were also examined in the cancerous tissues and in the surrounding gastric mucosa, and its implications in carcinogenesis were discussed.

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MATERIALS AND METHODS

1. Minute cancers of human stomach

From the resected stomachs documented at our Institute in the past 10 years, we collected very small diffuse type cancers and adenocarcinomas. The cancers less than 5mm in diameter were referred to as "minute cancer". The minute cancers were those found at the endoscopic examination, and they were the second lesion incidentally found in the stomachs resected for the primary cancer and ulcerative diseases. Some biopsied tissues were labeled with ^{3}H-thymidine *in vitro*, processed for conventional autoradiography. Others were labeled with BrdU, processed for immunohistochemistry using anti-BrdU antibody. The cancer tissues and gastric mucosal sections, which were cut serially to study pre- and paracancerous lesions, were also immunohistochemically stained with PCNA antibody, for identification of proliferative cells. In these materials, we studied cell proliferation of gastric cancers and the gastric mucosa involved in chronic gastritis with or without intestinal metaplasia. Phenotypic expressions of gastric cancers, intestinal or gastric, were assessed with mucin histochemistry with Alcian blue, PAS and concanavalin A staining, and with immunohistochemistry using MUC2, MUC5AC, MUC6 and CD10. Amplification of c-erbB2, c-met and k-sam and expression of oncogenes and antioncogenes (c-ki-ras and p53) was examined in gastric cancer cells and in non-cancerous gastric mucosa.

2. Experimental signet ring cell carcinomas in dog stomach

In order to examine a mode of development of signet ring cell carcinomas, we induced the cancers in dog stomachs with oral administration of ENNG, according to the method of Kurihara, et al. Male beagle dogs (1 year old, body weight 11-16kg) were given 75 mg of ENNG (Nacalai tesque, Kyoto Japan) with food, every day for 8 months. Five to 10 months after the ENNG administration, dogs were killed. Before sacrifice, they received single or multiple injections of ^{3}H-thymidine and BrdU, with which cell proliferation of cancerous tissues and the background gastric tissues were studied, with autoradiographic and immunohistochemical methods.

RESULTS AND DISCUSSION

1. Intramucosal distribution of initial signet ring cell carcinoma

In the present study, it was found that tiny (very minute) signet ring cell carcinomas were distributed around the neck region of glandular tubules, and small signet ring cell carcinomas formed a layered structure, the upper and lower part of which were composed of typical signet ring cells, and the middle layer consisted of small round cells. The small round cells were confined to the neck region. The neighboring glandular tubules around the smaller signet ring cell carcinomas were not involved in intestinal metaplasia. A varying degree of inflammatory reaction was seen in the lamina propria, but no special change was seen. These findings indicate that signet ring cell carcinomas arise at the neck region of gastric glands, and there were no precancerous lesion for the development of signet ring cell carcinomas.

2. Initial lesions of signet ring cell carcinomas in dog stomach

In the antral mucosa of several dogs treated with ENNG, in which no tumorous lesion was noted macroscopically, we found several initial signet ring cell carcinomas. In this, a very small cancer was seen at the neck region of glands; typical signet ring cells with mucin were seen at the upper part of the pyloric mucosa, and around the neck region, clusters of small round cells were seen. The signet ring cell carcinoma appeared to arise from the prolifer-

ative cell zone of the gland. There were no precancerous lesions and the signet ring cell carcinoma must arise de novo.

3. Development of intestinal metaplasia and adenocarcinomas in human stomach

The autoradiographic and immunohistochemical study with BrdU and PCNA indicated that in the normal pyloric glandular tubules, the proliferative cell zone was confined to the neck region of glands, whereas it was distributed at the lower one third level of the completely intestinalized glandular tubules. A starting point of intestinal metaplasia was found to be the neck region of gastric glands. To elucidate the histogenesis of adenocarcinomas, we looked for smaller lesions. In 256 resected stomachs, 27 minute adenocarcinomas and 11 minute adenomas were detected. The gastric mucosa around the minute lesions was mostly intestinalized. However, the smaller the carcinoma, the less complete was the form of intestinal metaplasia, and the metaplasia was often under progression in many glandular tubules. The smallest adenocarcinomas consisted of a few, newly formed glandular complexes, confined to the neck region, whereas the upper and the lower part of the tubules were still lined by normal gastric cells. The smallest adenomas were also seen around the neck region. These findings indicated that the starting point of the gastric neoplasias is the proliferative cell zone at the neck region, as is the case of intestinal metaplasia.

4. Development of adenocarcinomas judging from mucin phenotypes

We randomly selected 43 intramucosal adenoarcinomas less than 1.5 cm in diameter, and their mucin phenotypes were studied using MUC2, MUC5AC, MUC6 and CD10. To our surprise, it was found that 23% of these early cancers were gastric type, 30% were intestinal type and the remaining 47% were mixed of gastric and intestinal phenotype. This indicates that more than a half of early gastric cancers were not derived from the intestinalized mucosa but from the proper gastric mucosa. This finding was consistent with the abovementioned finding that the adenocarcinomas occurred at the neck region of pyloric glandular tubules.

5. Differences in genetic changes between diffuse type cancers and adenocarcinomas.

C-erb and c-erbB2 amplification was found only in adenocarcinomas, whereas k-sam amplification was seen only in diffuse type cancers (incidence of amplification was 0.6%, 18% and 15%, respectively). On the other hand, c-met amplification was seen both in diffuse type cancers and adenocarcinomas (incidence was 12% and 15 %, respectively). p53 mutation, as revealed by immunohistochemistry using wild type p53 protein, was seen in diffuse type cancers and adenocarcinomas. In the diffuse type cancer, the expression of p53 protein was seen only in 5% of the intramucosal signet ring cell carcinomas, whereas in the advanced stages, about 40% of the signet ring cell carcinomas were positive for p53-protein. In case of adenocarcinomas, 40% of the cancers were positive for p53 protein both in the early and the advanced stages. These findings may imply that genetic pathways for cancer development may be different between diffuse type cancers and adenocarcinomas.

SUGGESTED LITERATURE

1. Lauren P. The two histological main types of gastric carcinoma: diffuse and so-called intestinal-type carcinoma. Acta Pathol Microbiol Scand (A) 1965;64:34-9.

2. Sugano H Nakamura K, Kato Y. Pathological studies on human gastric cancer. Acta Pathol Jpn 1982;32:329-47.

3. Kurihara M, Shirakabe H, Izumi T et al. Adenocarcinomas of the stomach induced in beagle dogs by oral administration of N-ethyl-N'-nitro-N-nitrosoguanidine. Z Krebsforsch 1997;90:241-52.

4. Sugihara H, Hattori T, Imamura Y et al. Morphology and modes of cell proliferation in earliest signet ring cell carcinomas in canine stomachs induced by N-ethyl-N'-nitro-N-nitrosoguanidine. J Cancer Res Clin Oncol 1991;117:197-204.

5. Taki K. Kuwabara N. Studies on histogenesis of the gastric carcinoma using minute cancers. Pathol Res Pract 1981;172:176-90.

6. Hattori T, Fujita S. Tritiated thymidine autoradiographic study on histogenesis and spreading of intestinal metaplasia in human stomach. Pathol Res Pract 1979;64:224-37.

7. Hattori T. Development of adenocarcinoma in the stomach. Cancer 1986;57:1528-34.

8. Kushima R, Hattori T. Histogenesis and characteristics of gastric type adenocarcinomas in the stomach. J. Cancer Res Clin Oncol 1993;120:103-11.

9. Tsujimoto H, Hattori et al. Amplification of growth factor receptor genes and DNA ploidy patterns in the progression of gastric cancer. Virchow Arch 1997;431:383-9.