IMMUNOFLUORESCENT STAINING OF GASTRIC MUCOSAL GLYCOPEPTIDE IN GASTRIC CARCINOMA OF DOGS AND RATS INDUCED BY N-METHYL-N'-NITRO-N-NITROSOGUANIDINE

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Immunohistological studies, using the fluorescein isothiocyanate-labeled antibody against gastric mucosal glycoprotein, were made during the development of gastric cancer, induced in dogs and rats by oral administration of N-methyl-N'-nitro-N-nitrosoguanidine (MNNG). In an early stage, the regenerative glands were lined by fluorescent mucus cells. Cancer cells of orderly glandular structure, produced in dogs, were devoid of fluorescence. Cancer cells of less differentiation, produced in rats during further advanced stage, were well fluorescent. The immunofluorescent profiles of such experimentally induced gastric carcinoma were found to be the same as those of human gastric adenocarcinoma.

The immunohistological findings on the distribution of gastrointestinal mucosa-specific glycoproteins in human gastric carcinoma have been reported in our previous papers.1-3 Among human gastric adenocarcinoma cells, the poorly differentiated ones were recognized to possess or secrete these glycoproteins, whereas the well-differentiated ones with orderly glandular structure were devoid of them. When the glandular structures became less orderly arranged, carcinoma cells seemed to produce these glycoproteins.

The high rate of induction of adenocarcinoma in the glandular stomach of experimental animals by oral administration of N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) has been reported by Sugimura et al.4,6,7 In the course of development of experimental adenocarcinoma, three types of glandular proliferation were distinguished; regenerative glandular hyperplasia, adenomatous hyperplasia, and adenocarcinoma.

The diagnosis of stomach cancer depends largely on histological characteristics, including cellular atypism. It seems that more objective criteria are in need for distinguishing the above changes. Attempts were therefore made to elucidate whether the immunofluorescent profiles of human gastric adenocarcinoma could hold true for experimentally induced ones and whether they are useful in analyzing the developmental stages.

Materials and Method

Male dogs (pointer), about one year old and weighing 10~15 kg, were fed on pellet diet (Dogmeal No. 2, Hokuetsu Shiro Institute, Niigata) and given water containing 167 μg/ml of MNNG. Dogs drank about 300 ml of liquid a day. They were killed on the 98th (three dogs), 303rd (three dogs), and 485th (two dogs) experimental day.

Male Wistar strain rats, 8~9 weeks old and weighing about 150 g, were fed a commercial diet (CLEA-2) and received 167 μg/ml of MNNG in the drinking water. On the 540th day, two
Rats were killed. MNNG was purchased from Aldrich Chem. Co., Milwaukee, U.S.A.

Fluorescein isothiocyanate-labeled antibody against porcine gastric mucosal glycoprotein and immunofluorescent tissue preparations from dogs and rats were prepared as described previously. The mucus cells of glandular stomach of these animals were found to fluoresce with this antibody as intensely as those of human gastric glands. The organ-specificity of this glycoprotein was ascertained in these animals, examining other normal tissues as a reference. The identity reaction between human gastric glycoprotein and that of a dog or a rat was also confirmed by the Ouchterlony assay; i.e., this glycoprotein was recognized to possess organ specificity but not the strict species specificity between human beings and these animals (pigs, dogs, and rats).

**Results and Discussion**

**Dogs:** On the 98th day, the gastric mucosa of the experimental dogs was atrophied and eroded. The base of erosion was replaced by granulation tissues. At their margins and bases, irregular glandular proliferation developed.

On the 303rd day, the downward growth of adenomatous hyperplasia was recognized (Photos 1 and 2). The regenerative glands extended into the submucosa or at least into the muscularis mucosae. They were mainly lined by tall, columnar, mucus cells. There was little cellular atypism, and mitotic figures were found only in restricted part. These cells were well fluorescent as those of normal and regenerative glands.

On the 485th day, the carcinoma cells of orderly glandular structures, infiltrating the mucosa through the muscularis mucosae, were recognized (Photos 3 and 4). Such adenocarcinomas were composed of atypical and branched glands which were lined by multilayered, hyperchromatic, atypical cells of various sizes, shapes, and polarities, as already mentioned by Saito et al. Mitotic figures were numerous. These adenocarcinoma cells were mostly devoid of fluorescence or fluoresced only faintly in part, as in the case of well-differentiated carcinoma cells of the human stomach.

**Rats:** The gastric cancer of further advanced stage was produced in rats. On the 540th day, adenocarcinoma cells of mucinousulocellulare were recognized in experimental rats (Photos 5 and 6). They were less differentiated than those of the dogs. Signet ring cells were also found abundantly. These cells invaded the muscular layer and extended to the serosa. These cells and secreted mucus were well fluorescent.

Thus, the immunofluorescent profiles of gastric carcinoma induced in animals were found to be the same as those of human gastric cancer.

Various investigators have used different criteria for the diagnosis and classification of neoplastic lesions of the glandular stomach in experimental animals. Some have relied on atypicality of the altered mucosa, and the others on the depth of infiltration. In our immunohistological studies, carcinoma cells of orderly glandular structure, which appeared in the early stages of adenocarcinoma, were scanty of fluorescence and, therefore, sharply distinguishable from the well-fluorescent cells of the glandular and adenomatous hyperplasia. This immunohistological technique might offer a means for diagnosis of such experimentally induced gastric carcinoma.

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**References**

MNNG-INDUCED GASTRIC CARCINOMA


EXPLANATION OF PLATE

Photos 1 and 2. Hematoxylin-Eosin and immunofluorescence stain, respectively, of glandular stomach of a dog killed on 303rd day. Regenerative glandular hyperplasia and adenomatous hyperplasia in lower part of the erosion is remarkable. Their regenerative glands are well fluorescent.

Photos 3 and 4. Hematoxylin-Eosin and immunofluorescence stain of glandular stomach of a dog killed on 485th day. Adenocarcinoma cells of orderly glandular structure, infiltrating the mucosa through the muscularis mucosae, are only faintly in part or scarcely fluorescent.

Photos 5 and 6. Hematoxylin-Eosin and immunofluorescence stain of glandular stomach of a rat killed on 540th day. Adenocarcinoma cells of less differentiation, infiltrating into the muscular layer and extending to the serosa, are well fluorescent.