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Carcinoembryonic Antigen (CEA) in N-methyl-N'-nitro-N-nitroso-guanidine-Induced Gastrointestinal Carcinomas of the Rats[1]

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= Abstract =Immunohistologic findings on carcinoembryonic antigen (CEA) and its serum level were compared in N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)-induced gastrointestinal malignant tumors. Of fifty-six malignant neoplastic lesions developed from 39 rats, only 10 carcinomas were CEA positive (10.7% of gastric and 31.8% of small intestinal cancers) in cytoplasm of tumor cells by peroxidase-antiperoxidase (PAP) staining method. Animals with CEA positive carcinoma showed the highest level of serum CEA than any other groups, but the difference was not statistically significant (0.964 ± 0.150 versus 0.883 ± 0.094). The above findings lead to the suggestion that experimental gastric carcinoma expresses lower CEA positivity than intestinal carcinoma, comparatively similar to the reported results in human cases.

Key words: ***N-methyl-N'-nitro-N-nitrosoguanidine, Experimental carcinoma, Gastrointestinal carcinoma, Immunohistologic carcinoembryonic antigen***

INTRODUCTION

Carcinoembryonic antigen (CEA) was originally extracted from human fetal intestine and colon cancer tissue by Gold and Freedman (1965). It was subsequently shown that its presence in serum is associated with varieties of benign and malignant lesions. Thereafter, Goldenberg *et al.* (1976) demonstrated CEA in 60% of colon cancer and 14% of normal colonic mucosa using indirect immunoperoxidase method on formalin fixed, paraffin embedded tissue. And Ahnen *et al.* (1982) reported that 89% of colonic cancer tissue exhibited CEA positivity by PAP method. Although elevated serum CEA level was shown in only a portion of colon cancer patients, more than 80% of colon cancer tissue was positively stained by CEA in most of the studies (Huitric *et al.* 1976).

N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) is known as a potent carcinogen to the upper gastrointestinal tract when administered to rat (Sugimura and Fujimura 1967; Sugimura *et al.* 1970), and this experimental carcinoma model has been used for the human gastric cancer studies based on its selectivity of gastric and duodenal mucosa, histologic similarity and adenoma-carcinoma sequence (Bralow *et al.* 1970; Park *et al.* 1980). However, the tissue expression of tumor marker has not been examined to extend the applicability of this model. The purpose of this paper is to find any similarities in regard with CEA production between human gastrointestinal cancers and experimentally induced ones.

MATERIALS AND METHODS

The carcinogen, N-methyl-N'-nitro-N-nitrosoguanidine, was administered to a total of 117 Sprague-Dawley rats weighing 150-200 g initially. The MNNG was diluted in drinking water at a concentration of 100 μg/ml and fed ad libitum for 28 weeks. Fifteen weeks after the cessation of MNNG administration, blood was drawn from the heart, and animals were sacrificed. All of the removed stomachs including tumor tissue were immediately fixed in 10% neutral formalin and the full histologic examinations were performed by histotopographic method. The serum CEA levels were determined by

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Table 1. Numbers of experimental animals

Category	Control	MNNG-administered group	
		Tumor developed	Tumor not developed
Total	21	39(56)*	78
Serum CEA level checked	16	38(55)*	74

*: ( ) denotes the total numbers of the malignant tumors.

Table 2. CEA positivity in gastrointestinal malignant neoplasms induced by MNNG

Lesion	CEA positive	CEA negative	Total
Gastric carcinoma	3(10.7%)	25	28
Intestinal carcinoma	7(31.8%)	15	22
Sarcoma	0(0.0%)	6	6
Total	10(17.9%)	46	56

radioimmunoassay method (Radioassay Systems Laboratories, U.S.A.). The paraffin embedded tissues were processed using DAKO PAP kit by a modified peroxidase-antiperoxidase staining method against CEA. Briefly, after blocking endogenous peroxidase activity with 3% hydrogen peroxide and rinsing in a tris-buffer solution, normal swine serum was applied. The sections were sequentially incubated with rabbit anti-CEA, swine anti-rabbit IgG and rabbit PAP complex, with intervening rinsing in tris-buffer solution. The aminoethylcarbazole with hydrogen peroxide was applied to colorize the antigen. The slides were counterstained with Mayer's hematoxylin and cover-slipped. Twenty-one rats, without administration of MNNG, were served as control animals. More detailed methods were described in the previous report (Han *et al.* 1985).

RESULTS

Among 117 rats to which MNNG was administered, 39 rats developed malignant neoplasms. As shown in Table 1, a total of 56 tumors developed in 39 animals; 28 were gastric carcinomas, 22 intestinal carcinomas and 6 sarcomas, and the number of the tumors in each rat ranged from one to six. The positivity of tissue CEA in 56 tumors based on their locations or histologic type is summarized in Table 2. Only 10.7% of gastric carcinomas and 31.8% of duodenal carcinomas were CEA positive.

Only one case of differentiated adenocarcinoma in small intestine showed a strong positive staining of CEA. In this case the positivity was mainly confined to the apical surface of carcinoma cells (Fig. 1) and necrotic portions. Histologically this tumor was heavily infiltrated with neutrophils, and desmoplastic reaction was meager. Nine other adenocarcinomas, either in stomach or in small intestine, showed weak to moderate intensity on the cytoplasm of carcinoma cells (Fig. 2). None of the 6 sarcoma cases was positive in this investigation. Table 3 shows the summary of serum CEA level in all experimental groups. Although the CEA positive carcinoma group showed the highest level of serum CEA, there was no statistically significant difference among serum CEA levels of control animals, animals with tumors, animals with CEA (+) tumors, animals with CEA (−) tumors and animals without tumors.

DISCUSSION

This study showed that the experimentally induced gastrointestinal cancers in rats revealed comparatively low positivity rate in tissue CEA than in the reported human cases. The range of positivity rates of CEA in human gastrointestinal cancers is fairly wide depending on the location of tumor and investigators. In colonic cancers the tissue CEA positivity was about 60 to 100% (Goldenberg *et al.*

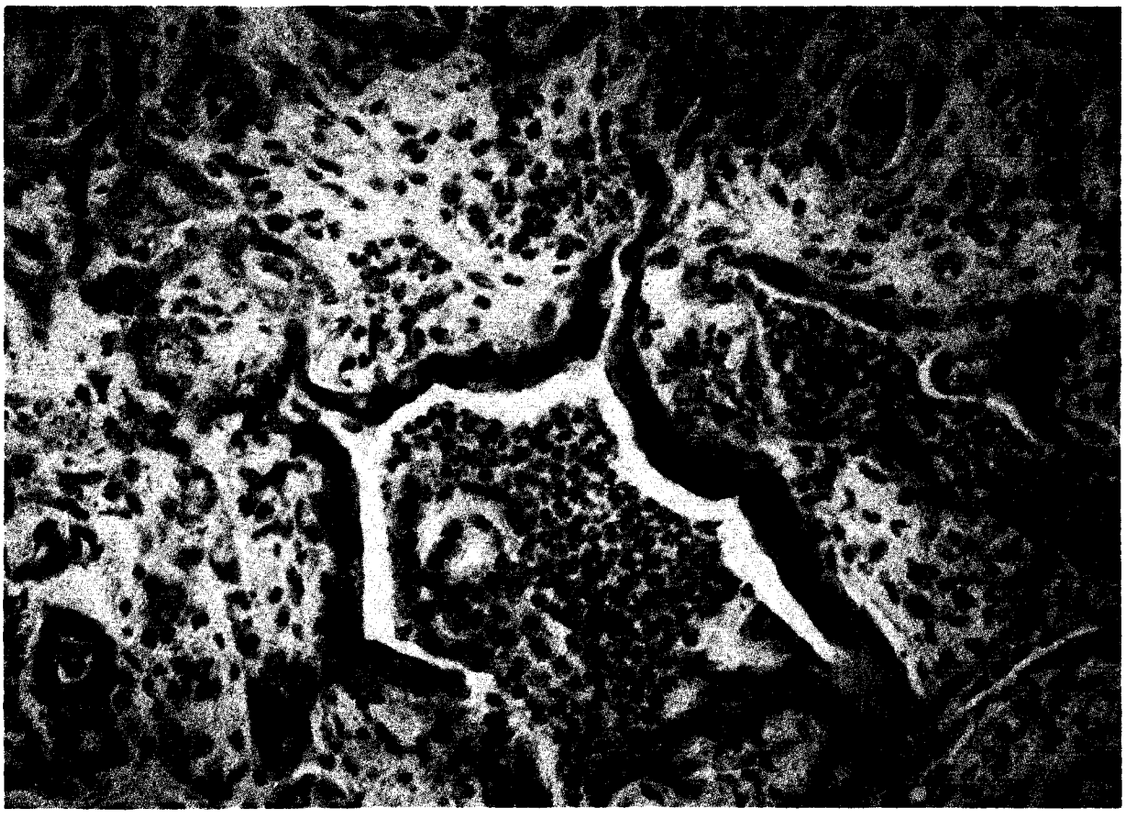

Fig. 1. Duodenal adenocarcinoma showing strong CEA positive staining in the cytoplasm. (PAP on CEA, × 200)

Fig. 2. Weak intesity of CEA positivity in adenocarcinoma of glandular stomach. (PAP on CEA, × 200)

Table 3. Comparison of serum CEA level with its tissue expression

Animal groups	No. of animals	Serum CEA**	
		mean	S.D.
Control animals	21(16)*	0.901 ng/ml	0.036 ng/ml
MNNG-administered animals	117(112)	0.900	0.101
with tumor(s)	39(38)	0.902	0.115
with CEA(+) tumor(s)	9(9)	0.964	0.150
with CEA(−) tumor(s)	30(29)	0.883	0.094
without tumor	78(74)	0.900	0.093

*: ( ) donotes the numbers of animals of which serum CEA levels were checked.
**: All values are not statistically significant.

1976; Huitric *et al.* 1976; Ahnen *et al.* 1982), while gastric cancer showed lower positivity rate (Goldenberg *et al.* 1976; Tanioku 1982; Kojima *et al.* 1984). In our observation, CEA positivity rate of small intestinal carcinoma is three times higher than that of gastric carcinoma, indicating comparably similar ratio to that of human cases.

Denk *et al.* (1973) and Burtin *et al.* (1973) found a characteristically uniform staining property in signet ring cell carcinomas and linitis plastica of stomach, and other histologic types failed to demonstrate such a uniform CEA positivity pattern. In this experiment, only one case of signet ring cell carcinoma was included (Kim *et al.* 1987), but this case was CEA negative. Neilsen and Teglbjaerg (1982) claimed that all 92 consecutive gastric cancer cases were positive in CEA staining using a two-layer unlabelled immunoperoxidase technique. He classified the positive reactions into 3 patterns and found a correlation between CEA staining patterns and histogenetic types. Since we used the PAP method which seems to be more sensitive than two-layer immunoperoxidase method, the difference of staining methods can be excluded as a major contributing cause for this low positivity, and discrepancy with our result should be evaluated along with a careful analysis of intensity. Furthermore, the tumor tissues were fixed in formalin for a few days in this study. As the CEA is glycoprotein, a delayed formalin fixation might be responsible for some of the false negative reaction in PAP staining. Difference of antibody affinity between human and murine CEA could be also another possibility of the above discrepancy.

The serum CEA level above 2.5 ng/ml is considered to be abnormal in human gastrointestinal cancers, but none of the rats in this experiment gave higher level than the above figure. Wagener *et al.* (1981) measured the tissue concentration of CEA in human gastrointestinal tumors and concluded that stomach cancer tissue harbors lower concentration than colorectal cancers, and only those ones with metastatic lesions exhibited high serum CEA levels. This may give an answer for a low CEA positivity and serum level in this study in which the cancers were totally confined within the stomach or the upper segment of small intestine and no single case showed lymph node or distant metastasis. Although small intestinal carcinomas demonstrated higher tissue CEA positivity than gastric ones in this MNNG model, it is required to compare with an additional experimental colonic carcinoma model to confirm its difference by the tumor location.

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= 국문초록 =

N-methyl-N'-nitro-N-nitrosoguanidine 투여에 의한 실험적 상부 소화관 암종의 암태생항원 발현에 관한 면역조직화학적 관찰

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흰쥐에게 실험적으로 유발된 상부 소화기암종 모형이 인체에서와 유사한 암태생항원 (CEA)을 발현하는지 여부를 알아보기 위해 N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)을 투여하여 39마리에 발생한 56개의 악성종양조직을 대상으로 peroxidase-antiperoxidase법에 따라 CEA의 검출을 시도하였다. 이중 총 10예에서 암조직내 CEA가 양성이었으며 부위별로는 위암의 10.7%, 소장암의 31.8%에 해당하였다. 이는 인체에서 위암의 CEA 양성 빈도가 장암에 비해 현저히 낮은것과 부합되는 소견이었다. 또한 각 동물에서 혈중 CEA의 농도를 측정하여 21마리의 대조군을 실험군과 비교하였던 바, 암조직내 CEA 표지 양성인 종양을 수반한 동물의 혈중 CEA 농도가 가장 높았으나 통계학적으로 의의있는 차이는 아니었다. 이상의 소견은 MNNG 투여에 의해 유발된 악성종양의 조직내 CEA 발현양상은 인체위장관암과 유사성이 있으나, 종양의 위치에 따른 혈청 및 조직내 CEA 양성율의 차이를 검정하기 위해서는 별도의 실험적 모델과의 비교 연구를 필요로 한다고 사료되었다.