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조기 위암에서 pS2 유전자 변형의 의의

장희경

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The significance of pS2 gene alteration in the early gastric cancer

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Abstract

Background and Purpose : The expression of pS2 gene in normal human tissue is restricted to the stomach. But its role in tumor development is not elucidated yet. The purpose of this study is to evaluate the functional role of pS2 protein in early gastric carcinomas(EGC), by investigating prevalence of the gene and analysing its expression with histologic types and differentiation. **Material and Method** : The formalin-fixed and paraffin-embedded tissue obtained from 100 patients with EGC who underwent gastrectomy by using immunohistochemistry. **Results** : In the non-neoplastic tissue adjacent to the tumor, the immunoreactivity for pS2 was observed in the cytoplasms of superficial and foveolar epithelium and a few chief cells, while intestinalmetaplastic epithelium showed negative immunoreactivity. The neoplastic tissue revealed cytoplasmic positive staining of tumor cells. The overall immunoreactivity for pS2 in 100 EGC cases is 56%. The expression of pS2 was significantly higher in diffuse type(65.9%) than intestinal type(48.2%) ($p<0.05$) and also higher than in poorly-differentiated type(62.1%) than in well-differentiated type(47.6%)($p<0.05$). The expression rates of tumors invading to mucosa was 53.1% (17/32) and tumors invading to submucosa was 46.9%(15/32). **Conclusion**: These results mean that the expression of pS2 is correlated to the histologic type and grading, while is not related to the depth of tumor invasion. And it is indicated that pS2 protein has a yet undescribed role in gastric foveolar epithelium and pS2 gene expression may be more important role in diffuse type cancer than in intestinal type cancer of stomach

Key words : pS2, Early gastric cancer, Lauren classification

INTRODUCTION

Stomach cancer has been the most cancer in Korea for many years. Even though there have been numerous studies about the oncogenesis, prevention and treatment. any stomach-specific oncogenesis has not reported yet. Recently pS2 gene was introduced by Rio.[1]. The pS2 gene encodes an 84-amino acid protein that is secreted after signal peptide cleavage[1]. It is specially expressed under estrogen transcriptional control in a subclass of estrogen receptor-containing human breast cancer cells[2]. The expression of pS2 gene in normal human tissue is restricted to and secreted by the mucosal cells of stomach and the concentration of pS2 peptide in gastric juice is 30-100 g/dl[1]. Lefebre[3]reported that mouse-pS2 is esstential for normal differentiation of the antral and pyloric gastric mucosa and may function as a gastric-specific tumor supressor gene. However the function of human pS2 protein in stomach is unknown yet,

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but in the development of gastric carcinomaaccording to Tahara[4,5]and Lauren.. Some reports that pS2 might involve the regeneration of peptic ulcer and chronic colitis[6]. Abnormal regeneration mechanism might explain partly the development of malignant tumor of gastrointestinal tract[7]. In breast cancer pS2 expression (mRNA) was considered as a significant predictor[8]. Thompson et al reported pS2 gene expression was correlated to recurrence of breast cancer, while especially in node negative patients pS2 expression indicates no mortality. But there is few study about pS2 associated with prognosis or clinical implication of gastric carcinoma[10]. Therefore, this study was attempted to evaluate the prevalence of pS2 by immunohistochemical method in early gastric carcinoma, to correlelate their expressions with histological types and differentiation of tumor, and eventually, to elucidate the significance of these proteins in gastric carcinoma.

MATERIALS AND METHODS

Material was 100 surgically resected, formalin-fixed, paraffin-embedded tissues of EGC obtained from patients who had underwent subtotal gastrectomy.

Immunohistochemical study

For immunohistochemical staining, 5㎛ thick sections of each specimen were mounted on positively charged slides and deparaffinized in xylene, and rehydrated through a decreasing concentration of ethanol. The antigen was retrieved in a microwave with 0.01M citrate buffer (pH 6.0) for 3-5 minutes. Following incubation with normal horse serum, the sections were incubated with 1:100 dilution of anti-pS2 polyclonal antibody (Norvocastra, England). Bound antibody was detected by the incubation of section for 30 minutes with biotinylated horse antimouse antibody (1:200 in TBS; Dako, Denmark). Prior to the addition of antibody, the slides were washed extensively in TBS.Color was developed with 3-amino-9-ethylcarbozole, and sections were counterstained with hematoxylin before mounting. The assessment of immunostaining was performed by the criteria of positivity defined as more than 10% of immunoreactivity of tumor cells. Positivity of pS2 was confined to cytoplasmic or perinuclear staining. Negative controls were run in parellel in which the primary antibody was replaced by non-immune rabbit antisera.

Classification of tumor

Histological classifications of EGC was performed on the basis of Lauren and cellular grading was accessed as well- and poorly-differentiated groups on the basis of WHO.

Statistical analysis

The statistical analysis was performed by chi-squered test, paired t-test and Kendall or Sperman tests were used to examine the relationship among the clinical parameters and gene expression. P value less than 0.05 were considered as significant.

RESULTS

Diffuse type was 44 (44.0%) and intestinal type was 56 (56.0%) out of 100 cases and well-differentiated type was 42 (42%) and poorly-differentiated type was 58 (58%) cases. In non-neoplastic gastric tissue adjacent to the tumor, the positive stainingwas seen in the cytoplasms of superficial and foveolar epithelium and a few chief cells, while intestinal metaplastic epithelium showed negative staining(Fig.1). The immunoreactivity for pS2 was 56% (56/100) (Table 1.) of 100 cases of gastric carcinomatissue. And their expression rates of mucosal tumors was 53.1% (17/32) and tumors the invading submucosa was 46.9%(15/32). It was not correlated with depth of tumor invasion (Table 2.). The diffuse type showed positive staining 29 out of 44 cases (65.9%) and intestinal type revealed 27 out of 56 cases (48.2%). The

positive cases in well-differentiated carcinoma were 20 out of 42 (47.6%) and in poorly-differentiated cases 36 out of 58 cases (62.1%). The expression of pS2 was significantly higher in diffuse type (65.9%) (Fig.2) than intestinal type (48.2%)($p<0.05$) (Fig.3) and poorly-differentiated type (62.1%) (Fig.2) than well-differentiated type(47.6%) ($p<0.05$) (Fig.3).

Table 1. Overall Expression of pS2 in 100 cases of EGC

	extent of expression				
	Negative	Positive			total
antibodies	0 - 10%	10-40%	41-70%	< 70%	expression(%)
pS2	44	40	14	2	56 (56)

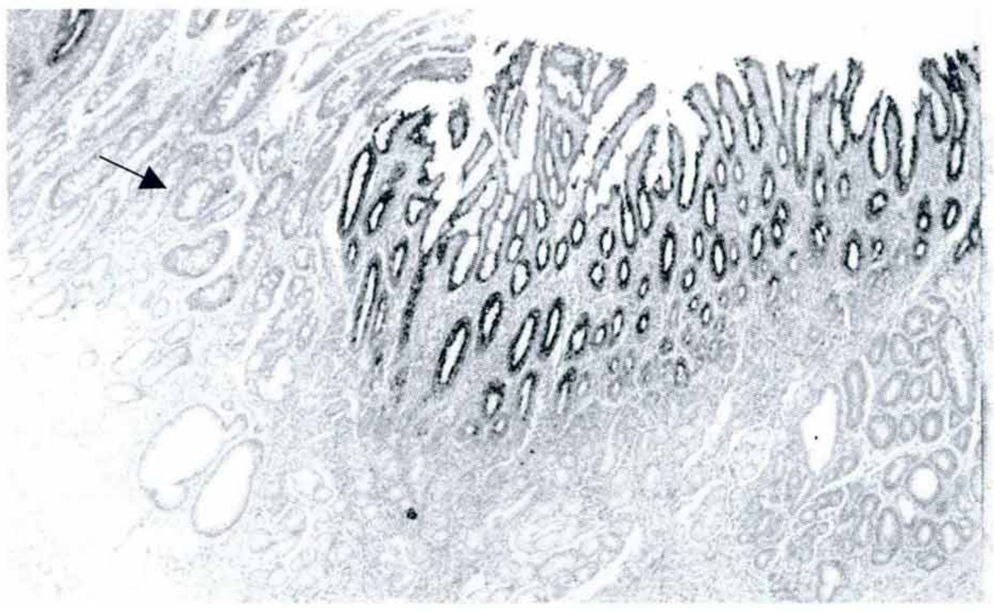

Fig 1. pS2 immunoreactivity of stomach; positive staining of the superficial epithelium and crypts, but no staining of intestinal metaplastic epithelium(->).(ABC, x40)

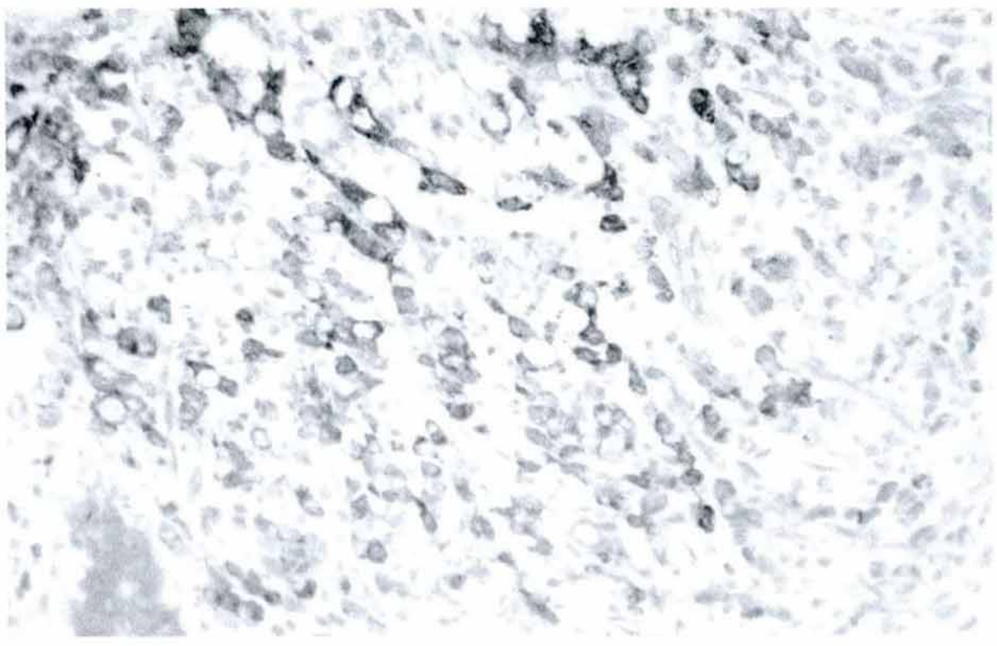

Fig 2. Strong positive immunoreactivity for pS2 in diffuse type carcinoma, poorly-differentiated Carcinoma (ABC, x200) (->: positive reaction)

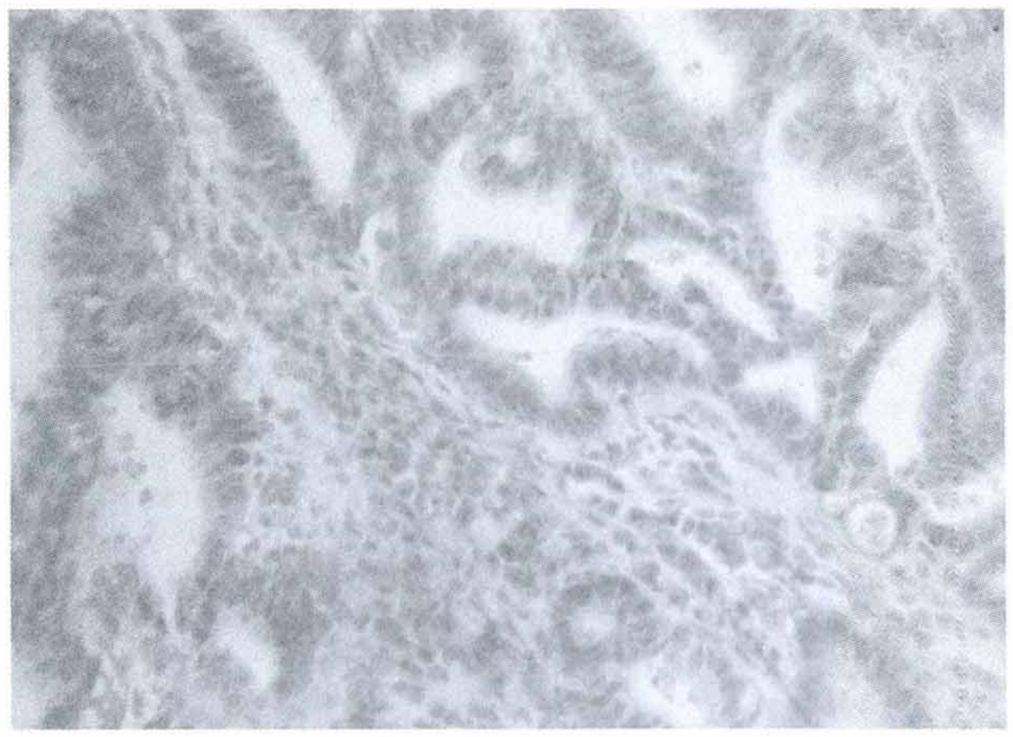

Fig.3 Focal weak positive reaction for pS2 in intestianl type cancer of stomach, well-differentiated. (ABC, x200)

Discussion

The human stomach appears to be the only normal tissue which expresses the pS2 gene[1]. Our observation also showed the negative staining of intestinal metaplastic areas, while positive staining was observed in the cytoplasms of normal superficial and foveolar epithelium. Luqmani et al [10] also suggested that pS2 is normally expressed in human stomach and becomes down-regulated during malignancy , as comparing with each mRNA level of normal, dysplasia, carcinoma, 100%, 70%, 18%, respectively. But the data of Welter et al [7] clearly indicated that 89% of colon cancer showed positive staining for pS2, while normal mucosa showed negative staining. Breast cancer were also reported that expressed pS2 protein[8]. Even though there is no expression of pS2 in normal, some malignancies express pS2 protein. We thought that pS2 might provide a certain clue to histogenesis of gastric type carcinoma of stomach. There has been a few studies in these field as yet. And we attempted this study to evaluate the significance of pS2 protein in the eagly gastric cancer tissue by investigating prevalence of both genes and their expression according to histologic types and differentiation, using immunohistochemistry on 50 cases of early gastric carcinoma. The immunoreactivity for pS2 in the present

study was 56%. Theisinger[11] reported that strong pS2 immunoreactivity was noted in the diffuse carcinoma type, whereas the intestinal type displayed week reactivity. But we could not recognized the significant difference of stainablity between two types of carcinomas. According to Mueller[12], pS2 immunoreactivity was 48% of 100 cases of gastric cancer, 32% of 9 cases of EGC. Our pS2 immumoreactivity was higher than Mueller's result. But Mueller's EGC cases seems to be not sufficient in number to evaluate its significance and to compare our results. .And its expression rate was not correlated with depth of tumor invasion (Table 2.).

Table 2. Expression of pS2 by depth of invasion in 100 cases of EGC

depth of invasion	No.	pS2 +	pS2 -
mucosa	32(32.0)	17 (53.1)	15 (46.9)
submucosa	68 (68.0)	39 (57.4)	29 (42.6)
total	100 (100.0)	56 (56)	44 (44)

P > 0.05 (percent)

The expression of pS2 was significantly higher in diffuse type (65.9%) than intestinal type (48.9%) ($p<0.05$) and poorly-differentiated type (62.1%) than well-differentiated type(47.6%) ($p<0.05$). Muller addressed that pS2 immunoreactivity was not correlated with tumor stage, grade, and Lauren classification [12].His results were not compatible with our results. These discrepancies might result from antibody or different epidemiology. The expression of pS2 was significantly correlated with that of p53($p<0.05$) (data not shown). In other words, there are significant co-expression of pS2 and mutant p53. Wild type p53 has been known as tumor supressor gene, and mutant p53 is suspected to be involved in the development of malignant tumor by the mechanism of loss of function of normal p53. However, the function and action mechanism of human pS2 protein in stomach is unknown yet, moreover in the development of gastric carcinoma. No major alterations of hpS2 have been found in genomic DNA extracted from gastric carcinomas.[10]. There is a report that pS2 gene expression was induced by mucosal ulcerations in the digestive tract, most notably in Crohn's disease and pS2 gene expression may provide a useful marker for mucosal ulcerations of the digestive tract[6]. But Lefebvre[3]reported that mouse-pS2 is esstential for normal differentiation of the antral and pyloric gastric mucosa and may function as a gastric-specific tumor suppressor gene. The gene profiling of normal and gastric cancer by Oien showed that pS2 gene was gastric specific gene[13]. Tahara addressed that more than 30% of intestinal metaplasia showed pS2 loss and speculated that the de-novo pathway for carcinogenesis of well- differentiated type carcinoma involves abnormal expression of p73 gene that are responsible for the development of foveolar-type gastric cancer with pS2 gene[14]. The current results, that. the positive staining was seen in the cytoplasms of superficial and foveolar epithelium and a few chief cells, while intestinal metaplastic epithelium showed negative staining in non-neoplastic gastric tissue adjacent to the tumorand the intestinal type gastric cancer showed significantly more cases of pS2 loss than diffuse type cancers, can support the Tahara's hypothesis. The further studies based on a larger series of cases, including investigations concerning the mode of activation of the gene and the protein function and survival analysis of patients would be needed to elucidate the functional significance or clinical implication of pS2 expression in the development of gastric cancer.

Conclusion

pS2 gene product seems to play a more crucial role in foveolar epithelium of normal stomach and pS2 gene expression may be more important role in diffuse type cancer than in intestinal type cancer of stomach. These

results have to be verified in further studies based on a larger series of cases, including investigations concerning the mode of activation of the gene and the protein function to elucidate the significance of pS2 in normal and neoplastic cells.

Table 3. Expression of pS2 according to Lauren classification in 100 cases of EGC

Lauren		antibodies
Classification	No.	pS2
Diffuse	44 (44.0)	29 (65.9)
intestinal	56 (56.0)	27 (48.2)
total	100 (100.0)	56 (100)

P < 0.05 (percent)

Table 4. Expression of pS2 according to differentiation in 100 cases of EGC

differentiation		expression
	No.	pS2
well	42 (42.0)	20 (47.6)
poorly	58 (58.0)	36 (62.1)
total	100 (100.0)	58 (100)

* ; P < 0.05 (percent)

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