

## Estrogen Receptor-related Protein(P29) in Gastric Cancer : Association with Histologic type and Stage

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= Abstract =

위암에서의 에스트로젠 관련 단백질의 표현 :  
조직학적 분류와 임상병기와의 관계

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Estrogen receptor-related protein was examined on gastrectomy specime from 16 cases of advaced gastric adenocarcinoma and 7 cases of early gastric carcinoma(EGC) by using peroxidase-anti-peroxidase method on formalin-fixed paraffin-embedded sections. Positive reaction was seen in 7 out of 16 cases of advanced carcinoma and in 4 out of 8 cases of EGC(50%). Among advanced carcinoma, 3 cases of mucinous carcinoma were negative and 2 cases of signet ring cell carcinoma(SRC) showed focal positive reaction only in combined poorly differentiated(PD) area(10% of tumor cells). PD advanced carcinoma consisted of 4 cases of medullary type and 3 cases of individual cell type. Two out of 4 medullary type showed positive reaction in 20 and 80% of tumor cells and 2 out of 3 individual cell type showed positive reaction in 50 and 70% of tumor cells. Gland-forming type of advanced carcinoma consisted of 1 each case of intestinal and cardiac type and 1 mixed intestinal and cardiac type. Only 1 case of intestinal type showed positive reaction in 50% of tumor cells. Among EGC, 2 cases of SRC were negative and 2 cases of PD carcinoma showed 5 and 10% positivity in PD area and 20 and 40% positivity in admixed gland-forming area. Gland-forming EGC consisted of 3 cases of intestinal type and 1 case of cardiac type. One case from each group showed positive reaction in 50 and 20% of tumor cells, respectively. In summary, positive reaction to antibody to estrogen receptor-related protein(P29) was expressed in PD(66.7%), gland-forming(50%), SRC, and mucinous type in order in both early and advanced carcinoma. The difference between age, sex, and other factors was not clear due to limitation of specimen.

**KEY WORDS** : Estrogen receptor-related protein(P29) · Stomach cancer · Early gastric carcinoma · Advanced gastric carcinoma.

## Introduction

The presence of estrogen receptor has been reported in non-sex hormone dependent tumors including malignant melanoma, kidney and brain tumors, gastrointestinal cancers, lung cancers, and others<sup>1-5)</sup>, which suggest that these tumors may be hormone-dependent or influenced by hormonal factors.

The incidence of gastric adenocarcinoma in wo-

men is as low as 50% of that in men<sup>6)</sup> and undifferentiated or diffuse type of gastric carcinoma is more common in younger female patients compared to intestinal type of adenocarcinoma<sup>7-8)</sup>. These findings suggest that sex hormones, particularly female hormones may play a role in regulating the growth of gastric cancer. Recently, sex hormone receptors have been studied in gastric cancer tissue<sup>9-18)</sup>, showing positive reactions for estrogen receptor (ER) and progesterone receptor (PgR) more frequently in Borrmann type IV and diffuse type with

Table 1. Advanced carcinoma

	Sex	Age	Borrmann type	Depth of invasion	LN	Histology	ER(%)
Mucinous type							
S91-436	M	72	III	PG	+		-
						+SRC	-
S91-2289	F	54	III	PG	+		-
						+SRC	-
S90-1614	F	49	III	PG	+		-
						+SRC	-
SRC							
S91-1103	F	68	III	M	-		-
						+PD	+(10%)
S91-2127	M	68	III	PG	+		-
						+PD	+(10%)
S91-2219	M	53	IV	PG	+		-
						+PD	-
PD(medullary)							
S91-2333	F	64	III	M	+		-
S91-328	M	56	III	PG	+		+(20%)
						+SRC	-
S89-3064	M	57	U	PG	+		-
S89-2817	F	32	U	PG	-		+(80%)
PD(individual)							
S89-3041	M	50	U	PG	+		+(70%)
S89-2505	M	37	EGCA IIc- like	M*	-		+(50%)
						+SRC	-
S89-1999	F	47	IV	PG			-
Gland forming WD to MD							
S90-4143	F	75	III	PG	+	WD, inte & card	-
S91-1372	M	57	III	M	-	WD, card	-
S90-3076	F	60	III	M	+	Inte	+(50%)

LN : lymph node, P : perigastric, M : muscle, M\* : superficial inner muscle laver, inte : intestinal, card : cardiac, U : unknown

scirrhous growth pattern.

The current study evaluates the expression of estrogen receptor-related protein(ErP) on advanced and EGC to see the difference between stages and various histologic types of the gastric adenocarcinoma.

### Material and Method

Gastrectomy specimen of 16 cases of advanced gastric carcinoma and 8 cases of EGC were obtained from Department of Pathology of Ewha Womans University Hospiatl. Advanced carcinoma cases included 8 males and 8 females and the age was 32~75 years old with the mean age of 56.2. The cases were summarized in Table 1. There were 3 cases of mucinous and SRC type each, 4 cases of medullary type PD carcinoma, 3 cases of individual cell type PD carcinoma, and 3 cases of gland forming well(WD) to moderately well differentiated (MD) carcinoma. All 3 cases of mucinous carcinoma showed areas of SRC carcinoma and all 3 SRC carcinomas were mixed with areas of PD carcinoma. Two cases of PD carcinoma were also mixed with SRC area. Three cases of gland-forming

carcinoma consisted of 1 each case of cardiac and intestinal type and 1 mixed intestinal and cardiac type.

The EGC cases included 2 males and 6 females and the age distribution was 45~69 years old with the mean age of 58.5. There were 2 cases of SRC type, 2 cases of individual cell type of PD carcinoma, and 4 cases of WD to MD gland forming carcinoma(Table 2). One case of SRC was mixed with PD area and the other with intestinal type WD carcinoma. Both cases of PD cases were mixed with gland forming areas. Four cases of gland forming WD to MD carcinoma consisted of 3 cases of intestinal type and 1 case of cardiac type.

Representative sections from each case were stained with monoclonal antibody to ErP(P29) by peroxidase-anti-peroxidase method described by Sternberg et al<sup>20</sup>. The results were interpreted in a semiquantitative manners based on the patterns of cell staining as negative(-) or positive(+), the latter with percentage of positively reacting tumor cells.

### Results

The results are summarized in Tables 1 & 2. In

Table 2. Early gastric carcinoma

	Sex	Age	EGCA type	Depth of invasion	LN	Histology	ER(%)
SRC							
S90-332	F	51	IIC	M	-		-
						+PD	-
S91-3374	F	57	IIB	M	-		-
						+WD, inte	-
PD							
S91-1696	M	58	IIC	M	-		+ (10%)
						+gl(card)	+ (20%)
S90-400	F	69	IIC	SM	-		+ (5%)
						+gl(inte)	+ (40%)
Gland forming WD to MD							
S90-3690	M	45	IIB	SM	+(2/8)	inte	-
S91-196	F	69	IIC	M	-	inte	+(50%)
S90-1725	F	55	IIB	SM	-	card	+(20%)
S90-3953	F	64	IIB	M	-	inte	--

LN : lvmph node, M : mucosa, SM : submucosa, inte : intestinal, card : cardiac, gl : gland

advanced gastric carcinoma, 7 out of 16 cases(43.7%) showed positive reaction. All 3 cases of mucinous carcinoma were Borrmann type III with infiltration into perigastric soft tissue. ErP was negative in all 3 cases in both mucinous and SRC areas. Three cases of SRC consisted of 2 cases of Borrmann type III and a case of type IV. Two cases were infiltrating into perigastric soft tissue and 1 into muscle layer. ErP was negative in signet ring cells and occasionally positive(10% of tumor cells) in admixed PD areas in 2 cases.

Four out of 7 cases(57%) of PD type showed 20~80% variable positivity of the cells. There was no significant difference in positive reaction between medullary type and individual cell type PD carcinomas. Four cases of medullary type PD carcinoma consisted of 2 cases of Borrmann type III and 2 of unknown gross classification. Three cases were infiltrating into perigastric soft tissue and 1 into muscle layer. Two cases were negative in ErP and 2 others showed positive reaction in 20% and

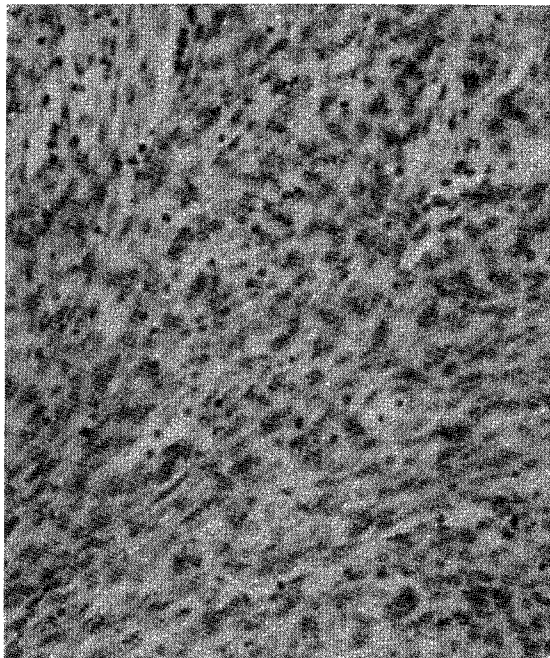


Fig. 1. Medullary type poorly differentiated advanced adenocarcinoma with positive reaction in 80% of tumor cells(S89-2817).

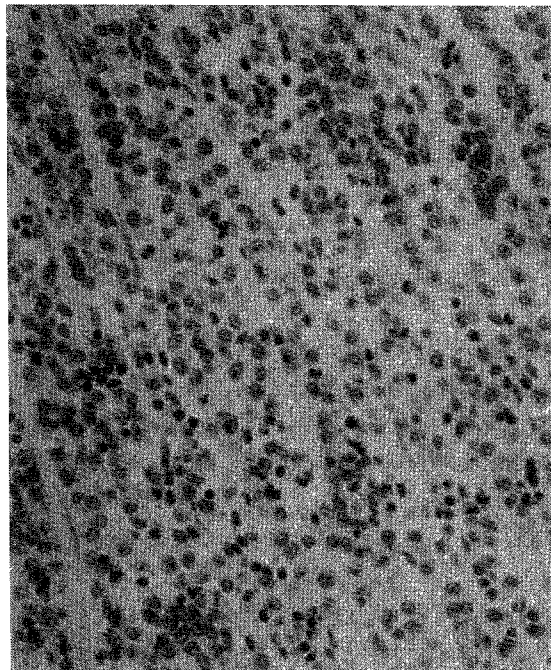


Fig. 2. Individual cell type poorly differentiated advanced adenocarcinoma with positive reaction in 70% of tumor cells(S89-3041).

80% of tumor cells, respectively(Fig. 1). The latter was 32 year old female and Borrmann type was unknown. Two of 3 individual cell type PD carcinoma showed positive reaction in 50% and 70% of the tumor cells, respectively(Fig. 2). One with 50% positivity was grossly EGC type IIc-like lesion with infiltration into superficial inner muscle layer. Mixed SRC area in that case was negative for ErP.

All 3 cases of gland-forming WD to MD carcinoma were Borrmann type III and infiltrating into muscle layer in 2 cases and preigastric soft tissue in 1 case. Only 1 case of interstitial type showed positive reaction in 50% of tumor cells.

Among EGC, 4 out of 8 cases(50%) showed positive reaction. Two cases of SRC type were EGC type IIc and IIb, respectively, with mucosal involvement. ErP was negative in signet ring cells as well as in admixed PD or gland-forming areas. Two cases of PD carcinoma were type IIc with mucosal and submucosal involvement, respectively. PD area showed 5 and 10% positivity compared to 40% and

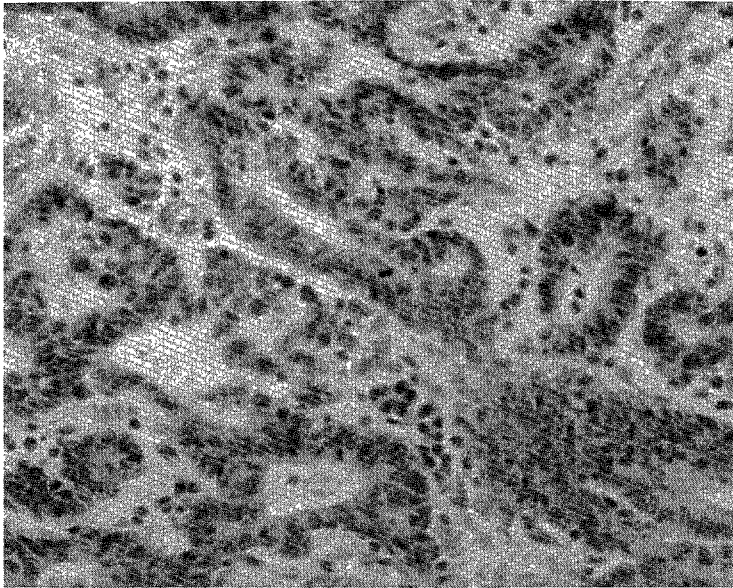


Fig. 3. Gland-forming area mixed with poorly differentiated early gastric adenocarcinoma with positive reaction in 40% of tumor cells(S90-400).

20% positivity in admixed gland-forming areas(Fig. 3). Gland-forming WD and MD EGC consisted of 3 cases of type IIb and a case of IIc and 2 each involved mucosa and submucosa, respectively. One case of 3 intestinal type showed 50% of positivity in tumor cells and 1 cardiac type showed 20% of positivity. One case with metastasis in perigastric lymph nodes was negative in ErP.

### Discussion

Gastric adenocarcinomas are usually divided into advanced and early carcinoma according to depth of invasion and into various histologic types based on pattern and differentiation of tumor cells<sup>19)</sup>. Difference in incidence and histologic types of gastric carcinoma according to age and sex of the patients has been suggested the hormonal or immunologic factors in this tumor<sup>7-8)</sup> and recently demonstration of hormonal receptors in tumor cells positively confirmed the relationship between these. Experimental study also showed hormonal influence on growth of gastric cancer in rats<sup>21)</sup> and culture of cell lines from ER-positive human gastric cancer

showed dependency on estrogen and suppression by antiestrogen<sup>22)</sup>.

The presence of estrogen receptors, located in nucleus, in gastric carcinoma was first reported by Tokunaga et al<sup>9)</sup> by dextran-charcoal method and immunohistochemical method was developed after that<sup>13-15)</sup>. Recently proteins regulated by, or related to, estrogen receptor were discovered in cytoplasm<sup>22)</sup> and proved to be a more reliable indicator of prognosis and hormone sensitivity than the demonstration of receptor itself. The p29, used in this study, is the monoclonal antibody to estrogen receptor-related protein recognizing a 29 Kd non-hormone binding protein associated with the estrogen receptor. Studies showed that this 29 Kd antigen is human-specific and present only in estrogen receptor-positive tissues such as breast, uterus and ovary<sup>23)</sup>.

In this study, overall positivity of ErP in advanced and EGC was similar(43.7% and 50%, respectively) but showed more positive reaction in PD carcinoma (66.7%) compared to gland forming WD to MD adenocarcinoma(50% of cases) or SRC or mucinous carcinoma(0%). These results were similar to previous studies. Tokunaga et al<sup>13)</sup> correlated ER

and PgR-positive cases with gross and microscopic characteristics of tumor and showed ER-positive cancers were grossly Borrmann type IV and microscopically diffuse type with scirrhous growth pattern. But they did not mention the relationship between Borrmann type and microscopic features or gross pattern. Also, histologic type was confined into two patterns only, intestinal and diffuse type according to Laurén classification<sup>19)</sup>.

Nishi et al<sup>15)</sup> did immunohistochemical study of intracellular estradiol(E2) in advanced gastric cancer and showed E2-positive tissue in 44% of 52 male patients and 20.6% of female patients with difference of expression according to age and histologic type. In their study, E2-positive cases occurred without age distinction in male compared to female with positive reaction only in younger age group. Also, male patients showed more positivity of E2 in intestinal type of cancer and female patients in scirrhous type of cancer. They showed the difference between ER and E2 with no definite correlation between them, and suggested occurrence of type II receptors due to difference in serum level of E2<sup>24)</sup>. Difference of E2 expression in different type of gastric carcinoma was obvious in their study, but it was not clear whether the presence of E2 in gastric cancer was due to production of E2 or not.

Recently, Harrison et al<sup>16)</sup> studied the effect of tamoxifen and estrogen receptor status on survival in gastric carcinoma. They used ERD5, estrogen receptor-related protein, and 53 of 95(55.8%) tumors were positive. Patients with ERD5-positive tumors showed significant decrease in survival time and tamoxifen therapy did not prolong survival. In addition, ERD5 showed fewer positive tumor cells in PD group(40%) compared to MD group(68%) and Laurén diffuse type showed fewer positive cells(29%) than intestinal type(69%). Their results were opposite to previous studies and this study, which suggested that positivity most likely depend on type of antibodies.

In this study, due to limitation of the specimen

number and cases, positive reaction according to age, sex, clinical stage was not evaluated. Further study with more specimen and correlation with proliferative activity of tumor cells are mandatory to decide the exact role and meaning of this protein in gastric cancer.

## Conclusion

In both EGCA and advanced carcinoma, positive reaction to antibody to estrogen receptor-related protein(P29) was expressed in PD, gland-forming, SRC, and mucinous type in order. As a result, it is presumed that estrogen receptor-related protein probably associated with proliferative activity of the tumor cells.

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= 국문초록 =

위암에서의 에스트로젠 관련 단백질의 표현 :  
조직학적 분류와 임상병기와의 관계

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진행성위암 16예와 조기위암 7예의 위절제술에 의한 표본에서 종양세포내의 에스트로젠 관련 단백질의 표현을 면역조직화학적 방법으로 검사하여 각 조직학적 유형간의 차이와 임상병기와의 연관관계를 관찰하였다. 진행성암의 경우에는 16예중 7예(43.7%)에서 그리고 조기위암의 경우에는 8예중 4예(50%)에서 양성반응을 보여 거의 비슷하였다. 각 조직학적 유형간의 차이는 진행성과 조기위암 모두에서 분화가 나쁜 경우에 가장 많은 양성반응을 보였고(66.7%) 분화가 잘되거나 중등도의 분화를 보이는 중앙에서 그 다음으로 많은 양성을 보였으며(50%) 인환세포종이나 점액세포종은 음성반응을 보였다. 이와 같이 분화의 정도에 따른 차이는 에스트로젠 관련 단백질이 종양세포의 증식능력에 관련이 있음을 시사한다.