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The Prognosis of Patients with Gastric Cancer Possessing Sex Hormone Receptors

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Abstract: Estrogen receptors (ER) and progesterone receptors (PgR) were immunohistologically investigated in 107 patients with gastric cancer who underwent curative resection. Both ER and PgR were detected only in the cancer cell nucleus. The ER positive rate was 27.7% for males and 31.0% for females, while the PgR positive rate was 9.2% for males and 11.9% for females. Clinicopathologically, the ER positive rate was slightly higher in young females and in cases of poorly differentiated gastric cancer. When cumulative survival rates were analyzed in relation to the presence or absence of receptors, the 10-year cumulative survival rate after surgery was significantly lower in the ER positive cases, being 15.7% cent, than in the ER negative cases, being 62.7%, and also significantly lower in the PgR positive cases, being 18.2%, than in the PgR negative cases, being 48.3%. The coexistence of ER and PgR in gastric cancer tissue suggests that the ER is physiologically active, or that ER positive gastric cancer is hormone-dependent. The poor prognosis of patients with receptor positive gastric cancer suggests that gastric cancer with these receptors is highly malignant.

Key Words: estrogen receptor, progesterone receptor, gastric cancer

Introduction

Sex hormone receptors have been examined as an index for judging the indications of endocrine therapy in patients with breast[1,2] or endometrial cancer[3,4] and the usefulness of this index as a prognostic factor has been assessed in various studies. Sex hormone receptors have been found not only in the target organs of sex hormones such as the breast and uterus, but also in cancers of such non-target organs as the stomach,[5,6] colon,[7,8] and liver.[9] Recently, monoclonal antibodies specific to estrogen receptors (ER)[10] and progesterone receptors (PgR)[11] have been developed and used for the histological study of breast cancer. Shimada et al.[12] reported that these receptors could also be localized using monoclonal antibodies in formalin-fixed, paraffin-embedded tissue sections. Following the recent detection of hormone receptors in gastric cancer, attention has been paid to the possibility of a hormone dependency of gastric cancer and the response of this cancer to endocrine therapy.[13]

In this study, we examined the immunohistological localization of ER and PgR in formalin-fixed, paraffin-embedded sections of gastric cancer in order to assess the hormone dependency and malignancy of gastric cancer. In addition, we analyzed the clinicopathological features of receptor positive gastric cancer and investigated the relationship between these receptors and the prognosis of gastric cancer patients.

Materials and Methods

Patients and Specimens

The subjects comprised 107 patients with gastric cancer who underwent a curative operation at the First Department of Surgery, Kyoto Prefectural University of Medicine. There were 65 males and 42 females. Excised tumors were fixed in 10% formalin at room temperature for 12–24 h, then embedded in paraffin by the routine method.

Immunocytochemical Staining Procedure for ER and PgR

Sections of 4 μm were cut, placed on slides, dried at room temperature overnight, deparaffinized in xylene,

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dehydrated, and rinsed in 0.05 M TRIS buffer with a pH of 7.4 at 24°C. The sections were then treated with several drops of DNase I (Sigma #D-5025) solution using 5 mg/ml of 0.05 M TRIS buffer, pH 7.4 at 25 °C and 0.01 M magnesium sulfate and incubated in a moist incubation chamber for 2 h at room temperature. Sections were then rinsed in TRIS buffer and incubated for 20 min with a blocking reagent using 2% normal rabbit serum in TRIS buffer (Vectorstain, Vector Lab.) to reduce the nonspecific binding of subsequent reagents. After gentle rinsing in TRIS buffer, the sections were incubated with several drops of a primary antibody using 0.1 μg/ml of H222 for ER (ER-ICA kit. Abbott Lab.) and 15.0 μg/ml of MPRI for PgR (Cosmobio. Lab.) overnight at 4°C in a moist incubation chamber. Sections were rinsed in TRIS buffer, incubated with biotinated rabbit antibody to rat IgG (50 μg/ml, Vectorstain, Vector Lab.) at room temperature for 120 min, then incubated with avidin-biotin peroxidase complex (Vectorstain, Vector Lab.) at room temperature for 120 min. After the final wash, sections were incubated in the dark for 5 min with the DAB solution, comprised of 20 mg of 3-3′ diaminobenzidin tetrahydrochrolide +10 μl of 30% H_2O_2 in 100 ml of 0.05 M ammonium acetate/citric acid buffer with a pH of 5.5–6.0. Sections were then washed in tap water, counter-stained with hematoxyline, dehydrated and mounted for examination by light microscopy. For each staining, a section from a specimen of human endometrial carcinoma was used as a positive control. A tumor was considered "positive" if any cancer cells were stained.

Statistical Analysis

The data obtained were evaluated by the χ^2 test. The survival data were calculated by the Kaplan-Meier method[14] and statistical analysis was carried out by generalized Wilcoxon tests to evaluate the significance of differences; *P* values of less than 0.05 were considered to be significant.

Results

ER and PgR Staining of Gastric Cancer (Fig. 1)

Immunocytochemical staining revealed ER and PgR in the nuclei of the tumor cells. The intensity of chromatic response, indicative of ER and PgR, was heterogeneous in individual cells from a given specimen.

Distribution of ER and PgR

Table 1 shows the ER and PgR positive rates. The ER positive rate was 29.0% for all cases, being 27.7% for males and 31.0% for females while the PgR positive rate was 10.3% for all cases, being 9.2% for males and 11.9% for females. There was no significant sex related difference in either positive rate.

Table 2 shows the background factors in relation to the presence or absence of RE and PgR. The mean age, histological stage of cancer and incidence of lymph node metastasis did not differ according to the presence or absence of receptors.

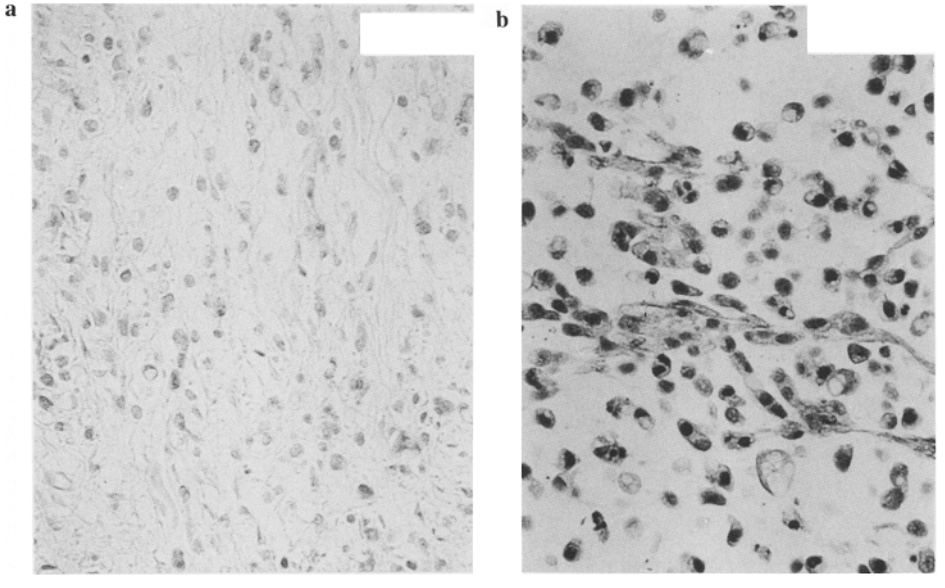

Fig. 1. a Estrogen receptor (ER) and **b** progesterone receptor (PgR) were localized in the nuclei of carcinoma cells. ER- and PgR-positive cells showed considerable heterogeneity with respect to the intensity of nuclear staining within the same section (×284)

Table 1. The distribution of estrogen receptors (ER) and progesterone receptors (PgR) in gastric cancer

	ER		PgR	
	(+)	(−)	(+)	(−)
Male (65 cases)	18 (27.7%)	47 (72.3%)	6 (9.2%)	59 (90.8%)
Female (42 cases)	13 (31.0%)	29 (69.0%)	5 (11.9%)	37 (88.1%)
Total	31 (29.0%)	76 (71.0%)	11 (10.3%)	96 (89.7%)

Relationship Between Age and Receptor Positive Rate

In females, the ER positive rate was highest in younger patients, being 100% in those aged below 39 years, 44.4% in those aged between 40 and 49 years, 36.4% in those aged between 50 and 59 years, 20.0% in those aged between 60 and 69 years and 16.7% in those aged over 70 years, whereas the PgR positive rate was highest in patients aged between 40–59 years (Fig. 2). In males, no age related difference was noted in either positive rate (Fig. 3).

Histologic Types and Receptor Positive Rate

The histologic types of gastric cancer in the 107 cases, classified according to the General Rules for the Gastric Cancer Study in Japan, were analyzed in relation to receptor positive rates. The ER positive rate was low in the 53 well-differentiated cases, being pap, tub1 or tub2 types and high in the 54 poorly-differentiated cases, being por, sig or muc types ($P < 0.01$). The number of PgR positive cases was small, and there was no significant difference in the PgR positive rate between the well-differentiated and poorly-differentiated cases (Fig. 4).

Receptor and Prognosis

Figure 5 shows the cumulative survival rates for the ER positive and negative cases after surgery. The 10-year survival rate was significantly ($P < 0.01$) lower for the ER positive cases, being 15.7% than for the ER negative cases, being 62.5%. The prognosis was therefore poor for ER positive gastric cancer.

Figure 6 shows the cumulative survival rates for the PgR positive and negative cases after surgery. The 10-year survival rate was significantly lower ($P < 0.05$)

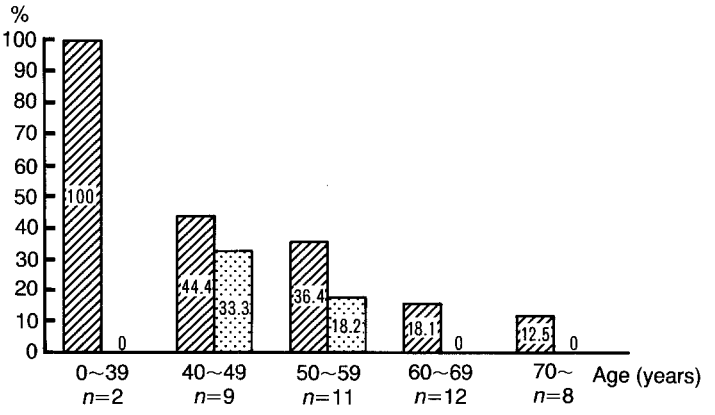

Fig. 2. Age and positive rate of estrogen receptor (ER) and progesterone receptor (PgR) in gastric cancer (female). *hatched area*, ER; *dotted area*, PgR

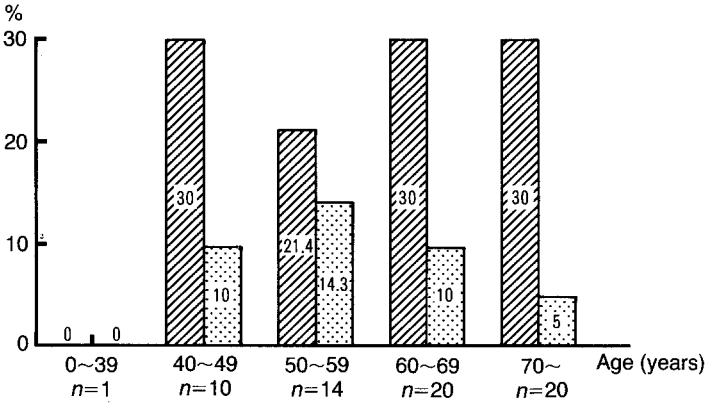

Fig. 3. Age and positive rate of estrogen receptor (ER) and progesterone receptor (PgR) in gastric cancer (male). *hatched area*, ER; *dotted area*, PgR

Table 2. Background factors of the gastric cancer patients

	Estrogen receptors		Progesterone receptors	
	Positive (31 cases)	Negative (76 cases)	Positive (11 cases)	Negative (96 cases)
Mean age (years)	60.3	60.1	57.8	60.4
Stage 1	13% (4/31)	16% (12/76)	27% (3/11)	21% (20/96)
Stage 2	16% (5/31)	16% (12/76)	10% (1/11)	16% (15/96)
Stage 3	45% (14/31)	49% (37/76)	46% (5/11)	36% (35/96)
Stage 4	26% (8/31)	20% (15/76)	0% (0/11)	8% (8/96)
Lymph node metastasis	26% (8/31)	38% (29/76)	36% (4/11)	34% (33/96)

Histological stages were classified according to the General Rules for Gastric Cancer Study in Japan[23]

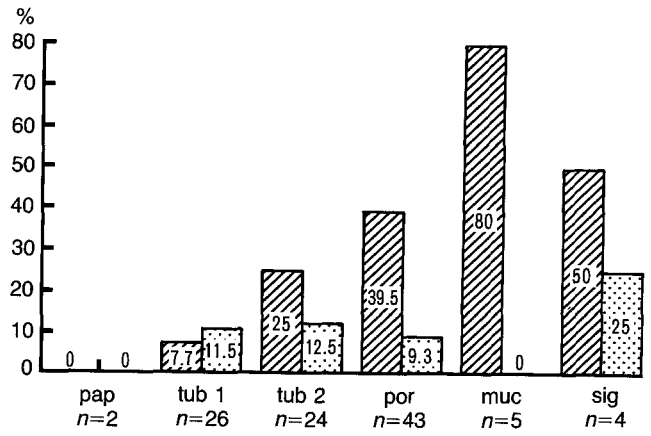

Fig. 4. The histological types of gastric cancer with positive estrogen receptor (ER) or progesterone receptor (PgR). The histological type was classified according to the General Rules for the Gastric Cancer Study in Japan. Well-differentiated type: pap + tubl + tub2; Poorly-differentiated type: por + muc + sig. *hatched area*, ER; *dotted area*, PgR

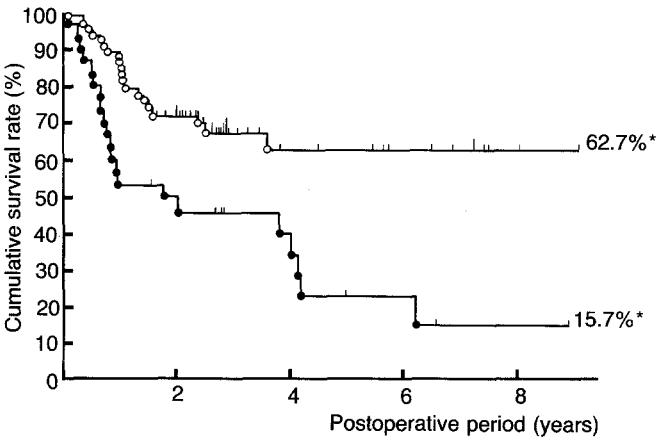

Fig. 5. Cumulative survival rate after surgery in patients with gastric cancer according to the stainability of ER (Kaplan-Meier method). *solid circles*, ER Positive (31 cases); *open circles*. ER Negative (76 cases). $^*P < 0.001$

for the PgR positive cases, being 18.2%, than for the negative cases, being 48.3%, and thus the prognosis for PgR positive gastric cancer was also poor.

Discussion

Tokunaga et al.[15] in a study on 86 cases of gastric cancer, reported the ER and PgR positive rates to be 15.4% and 9.6%, respectively, using the Dextran-Coated Charcoal (DCC) method. Recently, monoclonal antibodies specific to ER and PgR have been developed, and ER and PgR in breast and endometrial cancers have been detected using these antibodies.

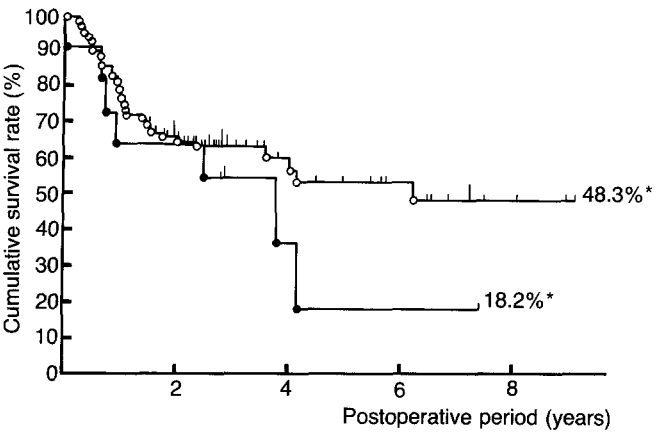

Fig. 6. Cumulative survival rate after surgery in patients with gastric cancer according to the stainability of PgR (Kaplan-Meier method). *solid circles*, PgR Positive (11 cases); *open circles*, PgR Negative (96 cases)

Kawakami et al.[16] reported that ER in breast and endometrial cancers could be detected using these antibodies even in formalin-fixed, paraffin-embedded tissue sections, and that the results of a test by the DCC method corresponded to those obtained from frozen specimens. In accordance with the findings of King et al.,[17] who observed these receptors in the nucleus of breast cancer cells, the present study disclosed receptors in the nucleus of gastric cancer cells. This agrees with the known biological significance of hormone receptors.

In the present study, the ER and PgR positive rates were 31.0% and 10.3%, respectively, thus, appearing to be higher when examined immunohistologically than by other methods. According to Yokozaki et al.[6] and Tokunaga et al.,[5] poorly differentiated gastric cancer showed a high ER positive rate. The present study supports this finding, also disclosing the positive rate to be higher in younger females, which suggests some relationship between the hormonal environment in females and the onset and growth of gastric cancer. Furthermore, the present study revealed clinicopathological differences between PgR positive and negative cases. Because the presence of PgR can be regarded as evidence of the biological activity of ER detected in gastric cancer, the present study supports the view that gastric cancer is dependent on hormones.

Under the assumption that the growth and development of cancer involves hormone receptors, it is likely that hormone receptors affect the prognosis of cancer. In this connection, some authors[18,19] found considerable differences in survival rates between patients with receptor-positive and those with receptor-negative breast cancers. According to Brocklehurst et al.,[20] the

prognosis of breast cancer was good in PgR cases and poorest in ER (+) PgR (−) cases. In this regard, the question arises as to whether or not hormone receptors correlate with the malignancy of gastric cancer. Harrison et al.[21] reported a poor prognosis for receptor positive gastric cancer, while according to Yokozaki et al.,[6] the prognosis of positive cases was evidently good in males but poor in females. Generally, ER positive breast cancer is thought to be less malignant than ER negative breast cancer because the degree of differentiation of ER positive breast cancer is closer to that of normal mammary tissue. In the present study, the prognosis for ER or PgR positive gastric cancer was significantly poorer than that for ER or PgR negative gastric cancer. The finding of hormone receptors in cancer of the stomach, which is not a target organ of hormones, can be interpreted as indicating that ER or PgR positive gastric cancer tissue is poorly differentiated compared to normal gastric tissue, and that the difference in the degree of differentiation from that of normal tissue is reflected in the malignancy of gastric cancer. Kitaoka[22] reported favorable results of endocrine therapy using tamoxifen, which was originally used for breast cancer, in females with scirrhous gastric cancer. We are also performing an open trial of endocrine therapy in patients with scirrhous gastric cancer, and have so far observed a tendency toward improved prognosis in ER positive cases. The present results therefore indicate that auxiliary endocrine therapy deserves further evaluation in ER or PgR positive cases of gastric cancer.

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