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Estrogen receptors in gastric adenocarcinoma: a retrospective immunohistochemical analysis*

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Summary. Estrogen receptors (ER) in human gastric carcinomas were examined immunohistochemically using a specific monoclonal antibody to human ER. ER-immunoreactivity (ER-IR) was positive in 30 (27.8%) of the 108 gastric carcinomas examined. ER-IR was located in the nucleus of cancer cells. The incidence of ER-IR positive gastric carcinoma was not significantly different between male and female cases. However, the positive tumour cells were observed in 28 (39.4%) out of the 71 poorly differentiated adenocarcinoma, the incidence being significantly higher than that in well differentiated adenocarcinoma ($p < 0.01$). There was no significant difference in the incidence of ER-IR between scirrhous carcinoma and non-scirrhous poorly differentiated adenocarcinoma. Synchronous expression of ER and epidermal growth factor receptor was found in 8 of the 26 scirrhous carcinomas (30.8%). Patients with ER-IR positive scirrhous gastric carcinomas showed a much worse prognosis than those with ER-IR negative scirrhous carcinomas.

Key words: Estrogen receptor – Gastric carcinoma – Immunohistochemistry

Introduction

Since the first description by Jensen et al. (1960), sex steroid receptors have been found in a variety of human tumours including brain tumours (Donnell et al. 1979; Kobayashi et al. 1982), lung cancer (Kobayashi et al. 1982), cancers of the alimentary tract (MacClendon et al. 1977; Kiang and Kennedy 1977; Kitaoka et al. 1982; Sica et al. 1982; Tokunaga et al. 1986), thymic tumours (Ranelleti et al. 1980), melanoma (Fisher et al. 1976) and pancreatic tumours (Greenway et al. 1981).

In these studies, dextran-coated charcoal (DCC) (Rosen et al. 1975) or sucrose density gradient centrifugation methods were used. Histological detection of estrogen receptors (ER) has been made using fluorescein immunochemical methods (Nenci et al. 1978; Pertschuk et al. 1978) or cytochemical methods, using directly conjugated estradiol or fluorescein bovine conjugated serum albumin (Dandliker et al. 1978; Lee 1978) and there are some discrepancies between biochemical ER assay and these cytochemical methods (Chamness et al. 1980).

Recently, a specific monoclonal antibody to human ER (Green et al. 1980; Miller et al. 1982) has provided a new era in the histological analysis of ER in human breast carcinomas. This monoclonal antibody is useful for ER immunohistochemistry on both frozen and sections paraffin and for comparisons with the biochemical assay (Shimada et al. 1985; Andersen et al. 1986). There has been no previous report on ER immunohistochemistry on gastric carcinoma tissues using this monoclonal antibody.

We have analyzed the presence of ER in human gastric carcinoma using this monoclonal antibody immunohistochemically and have examined the correlation of the results to histological type, fibrosis and prognosis.

Materials and methods

A total of 108 cases of gastric carcinoma were used. They were surgically resected and filed at the Department of Pathology, Hiroshima University School of Medicine. All were fixed in

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Table 1. Results of estrogen receptor immunohistochemistry in 108 gastric adenocarcinoma cases

Histological type[a]	No. of cases			ER positive cases		
	M	F	Total	M	F	Total
pap	3	1	4	0	0	0 (0%)
tub_1	3	1	4	0	0	0 (0%)
tub_2	19	10	29	2	0	2 (6.9%)
por	23	16	39	8	5	13 (33.3%)
sig	2	0	2	1	0	1 (50%)
muc	4	0	4	1	0	1 (25%)
scirrhous	13	13	26	8	5	13 (50.0%)

[a] According to the classification of Japanese Research Society for Gastric Cancer

10% buffered formalin and embedded in paraffin. One or two representative paraffin blocks were selected from each case for light microscopy and immunohistochemistry. In 8 cases of gastric carcinoma, the main tumour tissues were quickly frozen in liquid nitrogen for frozen section immunohistochemistry and enzyme immunoassay.

Histological classification of gastric carcinoma was made according to the criteria of the Japanese Research Society for Gastric Cancer (1985).

Rat monoclonal antibody to estrogen receptor H222 (Green et al. 1980) was purchased from Abbott Laboratories (North Chicago, USA). This monoclonal antibody has been shown to be ER-specific by several criteria, and cross-reactivity with other steroid receptors and cellular proteins is negligible (Miller et al. 1982).

Immunohistochemistry on paraffin sections was performed using the modified avidin biotin peroxidase (ABC) method after Andersen et al. (1986). Frozen section immunohistochemistory was performed using the ABC method and the peroxidase anti-peroxidase method according to the manufacturer's recommendations (Abbott Laboratories, North Chicago, USA).

In addition, the same specimens were immunostained with mouse monoclonal antibody to EGF receptor (EGF-R) (Oncor. Inc.; Gaithersburg, USA) which was developed using A431 epidermoid carcinoma cells as immunogen. The specificity of this monoclonal antibody to EGF-R has been described elsewhere (Yasui et al. 1988).

The immunoreactivities in tumour tissues were graded as follows: tissue with more than 50% of tumour cells having immunoreactivity was graded $+++$, 25 to 50% $++$, less than 25% $+$, and negative $-$.

For enzyme immunoassay for estrogen receptors frozen tissues were homogenized in 5 mM phosphate buffer (pH 7.5) containing 0.5 mM dithiothreitol and 10% glycerol and centrifuged at $105000 \times g$ for 90 minutes. The supernatant fraction was used for the assay. Estrogen receptor enzyme immunoassay (ER-EIA) kit was purchased from Abbott Laboratories (North Chicago, USA) and used according to the supplier's recommendations as follows: Beads coated with one monoclonal anti-ER antibody were incubated with the tumour cytosol or appropriate standards containing ER concentrations to cover the range from 0 to 500 fmol/ml. After aspiration and washing, a second monoclonal anti-ER antibody conjugated with horseradish peroxidase was incubated with the beads to measure the amount of bound ER. (3) After aspiration of the excess conjugate and washing, the beads were incubated with hydrogen peroxide and *o*-phenylenediamine. The intensity of the colour developed under this condition was read with a spectrophotometer at 492 nm. In each experiment, an additional control (lyophilized cytosol) was run as a check on the assay performance.

The data obtained were evaluated by χ^2 test and the difference of survival rates were calculated using Z-test.

Results

The incidence of ER immunoreactivity (ER-IR) in tumour cells of 108 gastric carcinomas is shown in Table 1. ER-IR was observed in 30 (27.8%) of the 108 gastric adenocarcinomas. The incidence

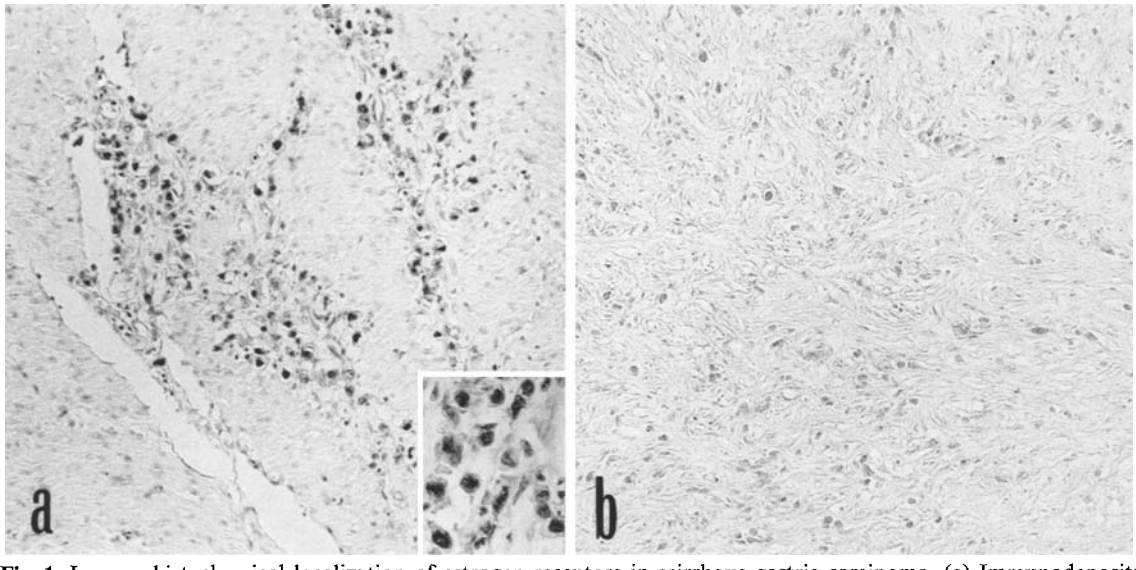

Fig. 1. Immunohistochemical localization of estrogen receptors in scirrhous gastric carcinoma. **(a)** Immunodeposits are observed at nuclei of diffusely infiltrating carcinoma cells. Paraffin section, avidin biotin peroxidase method ($\times 243$; *inset* $\times 486$). **(b)** Negative control ($\times 243$)

Table 2. Comparison of ER immunoreactivity between differentiated type and undifferentiated type gastric adenocarcinoma

Histology	Number of cases	Number of ER positive cases	
Well differentiated type[b]	37	2	a
Poorly differentiated type[c]	71	28	

[a] Significantly different ($p<0.01$)
[b] Including papillary adenocarcinoma and tubular adenocarcinoma
[c] Including poorly differentiated adenocarcinoma, signet ring cell carcinoma and mucinous adenocarcinoma

Table 3. Results of ER enzyme immunoassay and frozen immunohistochemistry of 8 surgically resected gastric carcinoma cases

Case number	Age	Sex	Histology[a]	EIA (fmol/mgprot.)	Immuno-reactivity[b]
1	71	M	tub_1	0.00	−
2	73	F	por (med)	20.89	+
3	65	M	tub_2	0.26	−
4	58	M	por (sci)	3.03	−
5	80	F	por (sci)	7.11	++
6	51	M	tub_2	1.18	−
7	50	F	por (sci)	3.68	−
8	54	M	tub_2	0.00	−

[a] According to the classification of Japanese Research Society for Gastric Cancer
[b] These reactions are graded +, ++ and +++ on the basis of the frequency of staining of individual cells

of ER-IR is not significantly different between male and female cases. The immunoreactivity in the ER-IR positive cases is mainly confined to the nucleus and perinuclear spaces in the tumour cells (Fig. 1). ER-IR was detected in 28 (39.4%) of the 71 poorly differentiated type gastric carcinomas including poorly differentiated adenocarcinoma, signet ring cell carcinoma and mucinous adenocarcinoma, whereas ER-IR positive tumour cells were detected in 2 (5.4%) out of the 37 well differentiated type adenocarcinomas including papillary adenocarcinoma and tubular adenocarcinoma. The difference of ER-IR between the two histological types was statistically significant by χ^2 test ($p<0.01$) (Table 2).

ER-IR was observed in 13 (50%) of the 26 scirrhous carcinomas, the incidence being not significantly different from that (33.3%) of non-scirrhous poorly differentiated adenocarcinomas.

Table 3 shows the results of ER enzyme immunoassay (EIA) and frozen section ER immunohistochemistry of 8 quickly frozen gastric carcinoma tissues. EIA value of two cases exceeded 5 fmol/mg protein. These two cases also had positive reaction for ER by immunohistochemical analysis (Fig. 2). The location of the immunoreaction for ER did not differ between ER-ICA kit staining and Andersen's modified ABC method in paraffin section.

A follow-up study was made on 23 cases of stages II and III scirrhous gastric carcinomas, and the 3-year survival rates were compared for patients with tumors positive and negative for ER-IR (Fig. 3). ER-IR positive patients had a poorer prognosis than those who were ER-IR negative

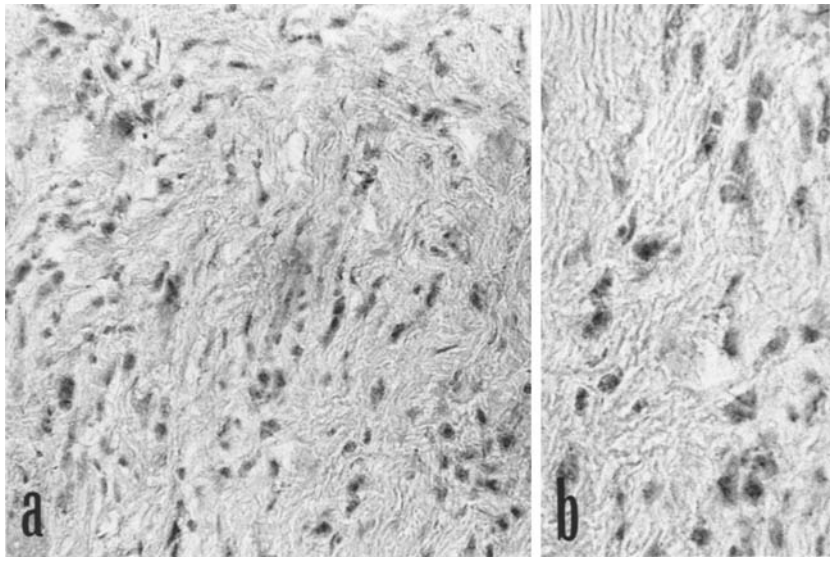

Fig. 2. Immunohistochemical localization of estrogen receptors in frozen section of scirrhous gastric carcinoma. Case No. 6 in Table 3. peroxidase antiperoxidase method (**a**) ×486, (**b**) (×800)

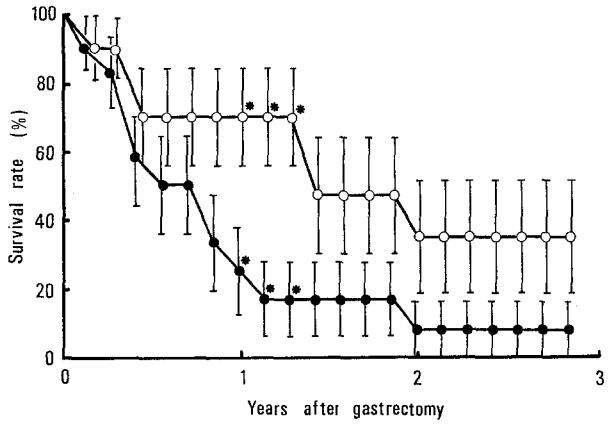

Fig. 3. Comparison of 3-year-survival rates in patients with stages II and III gastric carcinomas. ○; 11 cases (59±14.0 years old) without expression of ER. ●; 12 cases (57±15.2 years old) with expression of ER. The patients with ER positive carcinoma have poorer prognosis than those with ER negative cases and a significant difference become evident $1^1/_2$ years after gastrectomy (*; z-test, $p<0.05$). Bars, SD

Table 4. Comparison between estrogen receptor and epidermal growth factor receptor immunoreactivity in 26 scirrhous gastric carcinomas

	EGF-R[a] immunoreactivity	
	Number of positive cases	Number of negative cases
ER-IR[b] positive	8	7
	c	
ER-IR negative	0	11

[a] EGF-R: epidermal growth factor receptor
[b] ER-IR: estrogen receptor immunoreactivity
[c] significantly different ($p<0.05$)

and a significant difference was evident $1^1/_2$ years after gastrectomy (Z-test, $p<0.05$).

Comparison between ER and epidermal growth factor receptor (EGF-R) expression in 26 cases of scirrhous gastric carcinoma is shown in Table 4. A synchronous expression of ER and EGF-R was observed in 8 of the 26 scirrhous carcinomas (30.8%). Interestingly, EGF-R positivity was limited to ER-IR positive cases, no ER-IR negative case showed EGF-R immunoreactivity. The difference was statistically significant by χ^2 test ($p<0.05$).

Discussion

The existence of ER in gastric adenocarcinoma has been confirmed by several investigators using the dextran-coated charcoal (DCC) assay. The incidence of ER positive gastric carcinoma has been reported to be 10% by Kitaoka et al. (1982), 14.3% by Sica et al. (1984) and 15.4% by Tokunaga et al. (1986). We have found that ER-IR was located in the nucleus of gastric carcinoma cells on histological sections and that in all of the gastric carcinoma examined the overall incidence of ER-IR positive cases was 27.8%. The incidence obtained from histological sections was thus relatively higher than that by DCC assay. It is assumed that immunohistochemical analysis can detect scattered ER-IR positive cells which would not produce a positive biochemical assay, using homogenized tissue extracts. In fact, in our cases, the incidence of diffusely ER-IR positive gastric carcinoma was 10%, which corresponds closely to the previously reported figure.

Using DCC assay, the histological type of ER positive gastric carcinoma was predominantly poorly differentiated type adenocarcinoma (including poorly differentiated adenocarcinoma, signet ring cell carcinoma and mucinous adenocarcinoma Kitaoka et al. 1982; Tokunaga et al. 1986). We have also confirmed that poorly differentiated gastric adenocarcinoma showed a higher incidence of ER-IR than the well differentiated type with a statistically significant difference. In view of the stromal reaction in poorly differentiated type adenocarcinoma of the stomach, Tokunaga et al. (1986) reported that ER positive gastric cancers were characterized grossly as Borrmann type 4 and microscopically as diffuse type with a scirrhous growth pattern. However, we could not detect a significant difference in ER-IR positivity between scirrhous type cancers and non-scirrhous type cancers.

Epidemiological studies on gastric cancer show that the diffusely infiltrating or scirrhous gastric carcinoma is more common in females (Hirayama 1971; Stemmermann and Brown 1974). These findings might indicate that sex hormones, expecially estrogen, share an important role in the progression of scirrhous type gastric carcinoma (Tahara et al. 1982; Kitaoka and Tahara 1987). In this immunohistochemical study, ER-IR positive gastric carcinoma showed no female preponderance. Although there have been some reports on the effect of sex steroids in experimental gastric carcinogenesis, (Furukawa et al. 1982; Mandai 1987), it has not been clarified how estrogens affect the growth or progression of gastric carcinoma.

Recently, it has been reported that breast tumors or breast cancer cell lines expressing high levels of epidermal growth factor (EGF) receptors were generally found to be ER negative (Perez et al. 1984). Salomon and Perroteau (1986) have

suggested that breast carcinomas which are ER negative might contain a population of tumour cells whose growth is primarily regulated by growth factors such as EGF and not by estrogens. Conversely those tumours which are ER positive might possess a number of breast cancer cells that produce EGF or EGF-like peptides, such as TGF-α, whose synthesis might be under the control of estrogens. We have previously reported that 33.8% of gastric carcinomas showed EGF-R immunoreactivity (Yasui et al. 1988). In this study, we have observed a high incidence of ER and EGF-R coexpression in gastric carcinoma. The results contrast with those obtained from breast cancer. It may be assumed that EGF and estrogen play a cooperative role in the growth and progression of some gastric carcinomas and the expression of both receptors in tumour cells reflects a high grade malignancy.

There have been no reports on biological behavior, of ER-IR positive gastric carcinoma cases and here we have demonstrated that ER-IR positive scirrhous gastric carcinoma had a poorer prognosis than negative tumours. Kitaoka et al. (1982) reported the effectiveness of the anti-estrogen drug Tamoxifen on ER positive scirrhous gastric carcinoma. Immunohistochemical analysis may provide useful information on the question of whether a resected gastric carcinoma should be treated by anti-estrogen adjuvant chemo-endocrine therapy. More intensive study must be made, comparing the results of ER immunohistochemistry and biochemical ER assay, in order to determine the cutoff value of ER on immunohistochemical assay.

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