Estrogen and Progesterone Receptors in Gallbladder Cancer

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ABSTRACT: Cancerous tissues from 21 patients with primary gallbladder cancer were examined immuno-histochemically for the presence of receptors for estrogen (ER) and progesterone (PGR). ER and PGR, localized in the nucleus, were evident in 52.4 per cent and 0 per cent of the patients, respectively. Furthermore, ER and PGR were positive only in the cytoplasm of cancer cells in 28.6 per cent and 66.7 per cent, respectively. There was a higher tendency of moderately- and poorly-differentiated adenocarcinoma to have an ER-positive rate than well-differentiated adenocarcinoma. With respect to the relationship between ER and sex, ER-positive nuclei were observed in 8 of 14 women (57.1 per cent) and 3 of 7 men (42.9 per cent), but the difference between the two was not significant due to the small number of subjects. These result suggested that gallbladder cancers with ER in the nuclei may respond to antihormone therapy.

KEY WORDS: gallbladder cancer, estrogen receptor, progesterone receptor

INTRODUCTION

Estrogen-specific receptor (ER) is detectable in the cytosol of 60 to 75 per cent of all primary breast carcinomas.[1,2] In patients with estrogen receptor-positive breast cancer, hormone therapy is frequently effective as anticancer therapy.[1] Recently, the presence of hormone receptors in other tumors, including gastric cancer,[3,4] brain tumors[5,6] and neck tumors[7] has also been reported and some patients with the scirrhous-type of gastric cancer have appeared to respond to hormone therapy.[8] A high incidence of ER and progesterone receptors (PGR) in the mucosa of the gallbladder was reported recently after a biochemical assay,[9] however, there has been only one report involving three patients with cancer of the gallbladder.[10]

We conducted an immunohistochemical study to detect the presence of ER and PGR in carcinoma of the gallbladder. In this paper, we report our results and discuss the relationship of ER and PGR to the histological grade of gallbladder carcinoma. We postulate that the presence of ER and PGR in cancer cells of the gallbladder may furnish the biological basis to attempt endocrine therapy in the future.

MATERIALS AND METHODS

Tumor specimens from 21 patients with primary gallbladder carcinoma, who were admitted to the Second Department of Surgery, Hamamatsu University, School of Medicine, were used for this study. Specimens of gallbladder carcinoma were ob-

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tained following biopsy or surgery. The patients comprised seven men and 14 postmenopausal women.

Preparation of tissues and reagents

The tissues were fixed in 10 per cent buffered formalin immediately after collection and embedded in paraffin. Five μm paraffin sections were prepared.

Histokits for the immunohistochemical detection of ER and PGR were purchased from Biomeda Corp. (California, USA). ER and PGR were observed indirectly through the immunohistochemical detection of estradiol and progesterone combined with ER and PGR, respectively, using the Avidin-biotin-peroxidase system. The paraffin sections were deparaffinized and washed in phosphate buffered saline (PBS). In a brief summary of the immunological reactions using the Histokits, the tissues were incubated first with rabbit antiserum to estradiol or progesterone as the first antibody, then with biotinylated goat antirabbit immunoglobulin as the second antibody, and finally with avidin labelled with horseradish peroxidase. The 3,3′-diaminobenzidine tetrahydrochrolide solution with hydrogen peroxide was subjected to a color reaction. Tumor sections of breast cancer in which both ER- and PGR-positive cancer cells had been demonstrated biochemically and immunohistochemically, were used as a positive control in all cases. Those from which primary or secondary sera were removed were used as a negative control. The presence and localization of ER and PGR, stained immunologically, were examined by light microscopy.

Histologic findings of gallbladder cancer

The classification of the cancer by microscopy was performed according to Albores-Saavedra and Hensen.[11] Histologic types were based on hematoxylin eosin stained sections of the cancer. Twenty-one patients with gallbladder cancer were examined for ER and PGR with respect to their sex, histologic type of cancer and localization in the cancer cell.

Results

Estrogen receptor

The ER-positive cancer cells formed a mosaic pattern with the ER-negative cancer cells within the carcinoma. The intracellular localization of ER in the gallbladder cancer tissues could be classified into the three following types: Type I, located in the cytoplasm and nucleus (Fig. 1); Type II, located in the cytoplasm only (Fig. 2); and Type III,

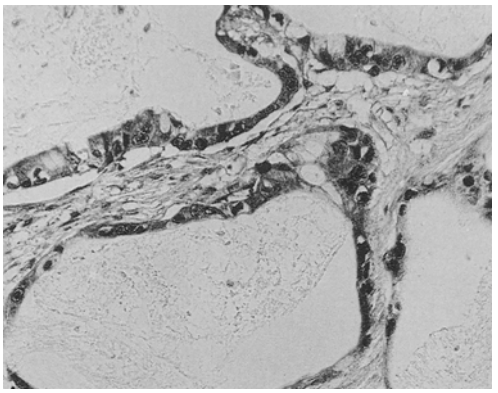

Fig. 1. ER localized in both the nuclei and cytoplasm of cancer cells of the gallbladder (Type 1). The histologic classification of this case is moderately differentiated adenocarcinoma.

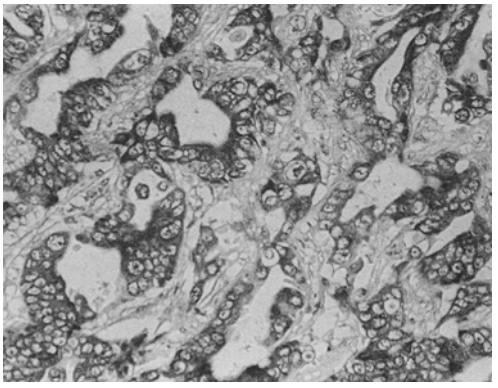

Fig. 2. ER localized in the cytoplasm of cancer cells (Type 2). This histologic classification is moderately differentiated adenocarcinoma of the gallbladder.

Table 1. Histologic Types and ER Localization

Histologic Types	ER Localization			Total
	N (+) Cy (+)	N (−) Cy (+)	N (−) Cy (−)	
Well-Diff. Adenoca.	4 (44.4%)	3	2	9
Mod.-Diff. Adenoca.	4 (50%)	3	1	8
Poorly-Diff. Adenoca.	2 (100%)			2
Poorly-Diff. Adenoca. with Squamous Metaplasia			1	1
Carcinosarcoma				1
Carcinomatous Elements[a]	1			
Sarcomatous Elements[b]			1[b]	
Total	11 (52.4%)	6 (28.6%)	5 (1[b]) (19.0%)	21 (1[a])

Abbreviations: N, nucleus; Cy, cytoplasm; a, moderately differentiated adenocarcinoma, b, spindle cell sarcoma.
Sarcomatous elements were not included in the total numbers of examinations.

ER-negative. Of the 21 patients, 52.4 per cent belonged to type I, 28.6 per cent to type II, and 19.0 per cent to type III (Table 1). There were no patients with ER localized only in the nucleus. ER-positive nuclei were observed in 8 of the 14 women (57.1 per cent) and 3 of the 7 men (42.9 per cent). In a comparison of the ER-positive ratio between the sexes, however, no significant difference was seen, due to the small number of patients. The presence of ER in the nucleus appeared to correlate with the histologic type. The positive rate of ER in moderately- and poorly-differentiated carcinoma was higher than that in well-differentiated carcinoma (Table 1). In one patient with poorly-differentiated adenocarcinoma, most of which was accompanied by squamous metaplasia, ER was negative in both the nucleus and cytoplasm. In a patient with carcinoma of the gallbladder containing both spindle sarcomatous-type cells and moderately differentiated adenocarcinoma, ER-positive cancerous nests formed a mosaic pattern with ER-negative sarcomatous tissues.

Progesterone receptor

No PGR was detected in the nucleus of any case, however, the cytoplasm of the cancer cells was stained positively in 66.7 per cent of the 21 cases (Fig. 3). The PGR-positive cancer cells also formed a mosaic pattern with the PGR-negative cancer cells although a relation between PGR-positive staining and histologic type was not recognized. PGR-positive cytoplasm was recognized in the cancer cells of 71.4 per cent of men and 57.1 per cent of the women but this difference was not significant (Table 2).

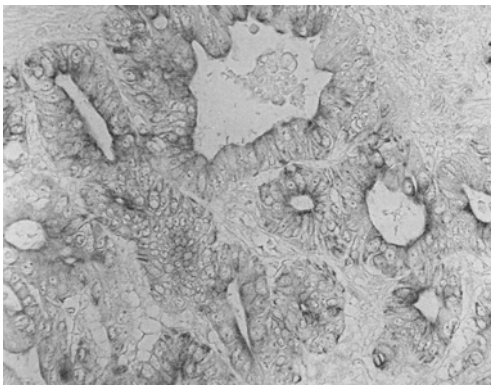

Fig. 3. PGR localized in the cytoplasm of cancer cells of the gallbladder. The histologic type is moderately differentiated adenocarcinoma.

Population of ER-positive cancer cells

Based on the percentage of cancer cells with ER-positive nuclei in the cancer cell population, the criteria of ER-positive levels were defined, according to Andersen,[12] as follows. If ER-positive cancer cells accounted for 50 per cent or more of the total number of cancer cells, the subject was assessed as ER (+); 10 per cent or less, ER (−); and in

Table 2. Histologic Types and PGR Localization

Histologic Types	PGR Localization		Total
	N (−) Cy (+)	N (−) Cy (−)	
Well-Diff. Adenoca.	6 (66.7%)	3	9
Mod.-Diff. Adenoca.	4 (50%)	4	8
Poorly-Diff. Adenoca.	2 (100%)		2
Poorly-Diff. Adenoca. with Squamous Metaplasia		1	1
Carcinosarcoma			1
Carcinomatous Elements	1[a]		
Carcinomatous Elements		1[b]	
Total	13 (66.7%)	8 (1[b])	21 (1[b])

See Table-1 for abbreviations.

Table 3. Histologic Types and the Population of Cancer Cells with ER-positive Nuclei

Histologic Types	Histologic ER			Total
	ER (+)	ER (±)	ER (−)	
Well-Diff. Adenoca.	4 (44.4%)		5	9
Mod.-Diff. Adenoca.	4 (50%)		4	8
Poorly-Diff. Adenoca.	1	1		2
Poorly-Diff. Adenoca. with Squamous Metaplasia			1	1
Carcinosarcoma				1
Carcinomatous Elements[a]	1[a]			
Sarcomatous Elements[b]			1[b]	
Total	10 (47.6%)	1	10 (1[b])	21 (1[b])

See Table-1 for abbreviations.

between these two groups, as intermediate, ER (±). Ten (47.6 per cent) of the 21 patients were ER (+) (Table 3), and only one patient with ER-positive nuclei was ER (±).

Discussion

The determination of ER in tumor tissues is an important diagnostic criterion in the management of patients with advanced breast cancer.[1] Tumors with an ER content of more than 10 fmol/mg cytosol and nuclear protein have been considered positive by biochemical assay, whereas specimens showing nuclear staining have been considered positive by histochemical assay.[13] The evaluation of ER by immunohistochemical methods, however, has been controversial for a long time. McCarty et al.[14] reported that the biochemical method is superior to the immunohistochemical method in terms of definition and localization of ER, since there were fewer positive cells demonstrated in comparable sections from each carcinoma. On the other hand, some authors[12,15–17] have demonstrated endogenous estrogen in formalin-fixed and paraffin-embedded tissue sections of tumors, using the PAP immunoperoxidase method. Consequently, an agreement between biochemical assay and immunohistochemical semiquantitative assays has been reported. Immunohistochemical methods have the advantage of being able to use the wealth of samples accumulated by many institutes during retrospective studies, however, when using such samples,[18] mul-

tiple sections should be examined because of the heterogeneity of the tumor tissue.[19] Since carcinoma of the gallbladder is an unusual disease, it is difficult to acquire many specimens for biochemical assay in a short period. Therefore, we studied ER and PGR in tumors retrospectively, by the immunohistochemical method, using specimens obtained over the last 9 years. The presence of ER and PGR in many different human tumors has been reported. Tokunaga et al.[3] reported that ER-positive stomach cancers were characterized grossly as Borrman type 4 and microscopically as diffuse type cancer with a scirrhous growth pattern composed of undifferentiated cancer cells. Sica et al.[4] and McClendon et al.[20] found ER in 22.8 per cent and 24 per cent, respectively, of the primary colon cancers they analyzed. The ER- and PGR-positive ratios seem differ in different organs. Recently, Stedman et al.[10] determined the ER- and PGR-protein values of diverse tumors and found high ER-protein values in 2 out of 3 patients with carcinoma of the gallbladder. Again, however, the number of subjects studied was few, and the immunohistochemical method was used. Our study confirmed the presence of ER in 52.4 per cent of patients with carcinoma of the gallbladder and showed a relationship between histologic grade and ER-positivity in the nuclei of the cancer cells. A high incidence of ER-positive cancer cells was observed in moderately- and poorly-differentiated cancers of the gallbladder. Furthermore, high ER-positive rates have been reported in poorly-differentiated carcinomas of the stomach and ovaries.[3,21]

There was no PGR in the nuclei, but PGRs were found in abundance in the cytoplasm in our study, although the significance of this finding is still unclear. It is possible that these PGRs are functional, since progesterone affects gallbladder contractility,[22] and PGRs are controlled by the estrogen receptor system in human breast tumor cells.[23]

The majority of patients with carcinoma of the gallbladder are older woman. The difference in the frequency between the sexes therefore reminded us of the correlation between steroid receptors and gallbladder cancers. Although we could not find a significant difference in ER- and PGR-positivity between men and women, this problem should be discussed by the determination of more specimens.

It has been reported that patients showing ER-positivity only in the cytosol had a lower response rate to endocrine therapy than patients showing receptor positivity in both the cytosol and nuclear fractions.[14] Moreover, McGuire et al.[1] has reported that 55–60 per cent of patients with ER-positive breast cancers achieved a remission with endocrine therapy. Our results suggest that adenocarcinoma of the gallbladder contains receptor sites for estrogen in the nuclei of the cancer cells and may also respond to hormone therapy.

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