Estradiol and Progesterone Receptors in Malignant Gastrointestinal Tumors

Vincenzo Sica, Ernesto Noia, Enrico Contieri, Rodolfo Bova, Maria Teresa Masucci, Nicola Medici, Antonella Petrillo, Alessandro Weisz, Anna Maria Molinari, and Giovanni Alfredo Puca

ABSTRACT

Estradiol and progesterone receptors were assayed in tumors from 79 patients with primary colorectal and 56 patients with stomach adenocarcinomas. Eighteen of 79 colorectal cancers contained estradiol receptor, while 34 specimens were positive for progesterone receptor. In stomach cancer, the positive samples were 8 for estradiol and 14 for progesterone receptors. In both types of tumors, the KD was in the range of 10^-10 M for estradiol and 10^-8 M for progesterone receptor, respectively. In colorectal adenocarcinomas, the presence of progesterone receptor seems to be partially correlated to the presence of estradiol receptor while, in stomach tumors, this correlation is lost. The positivity of at least one receptor in colorectal cancers is higher in the female sex. The contrary occurs for stomach cancer. Sucrose gradient centrifugation showed that cytoplasmic estradiol receptor of stomach cancer sedimented at 8S or 4 to 5S at low ionic strength. The isoelectric point of stomach cancer estradiol receptor is 6.5.

INTRODUCTION

The presence of steroid receptors has been well documented in hormonal-dependent cancers, such as breast cancer (15-17, 21, 22, 33), acute leukemia (18), prostate (14), and endometrial cancer (25). In addition, steroid-binding proteins have been identified in some malignant melanomas (10), kidney adenocarcinomas (8), and malignant skeletal tumors (32).

The association of breast cancer and extramammary malignant neoplasm has been described (11). The predominant site of the extramammary cancer is colorectal. Breast cancer can precede, follow, or even be synchronous with gastrointestinal carcinomas (11). Schoenberg et al. (29) and Berg and Foote (4) reported several primary lesions discovered at autopsy.

Common etiological factors associated with the 2 primary sites have been noted (3, 9, 12, 13). Recently, the presence of hormone receptors for estradiol, progesterone, dihydrotestosterone, and glucocorticoid has been described (1, 20) in tumors with patients with primary colon cancer.

ER2 in this neoplastic tissue has also been characterized (30, 31).

In this paper, we report our results on the presence of ER and PGR in cancers of the gastrointestinal tract. Molecular parameters and other properties of ER were investigated. Furthermore, we postulate that the presence of ER and PGR in these tumors may furnish the biological basis to attempt an endocrine therapy on these patients.

MATERIALS AND METHODS

Compounds. All reagents were of analytical grade. 17l-[^3H]Estradiol (specific activity, 85 to 115 Ci/mmol) and [3H]progesterone (specific activity, 80 to 110 Ci/mmol) were from Amersham. Dithiothreitol was from Calbiochem. Tris (Trizma base, reagent grade), EDTA (disodium salt), phenylmethysulfonfonyl fluoride, and pepstatin A were purchased from Sigma. 17l-Estradiol, progesterone, and hydrocortisone were from Calbiochem. Charcoal (Norit A) was from Matheson Coleman and Bell (Norwood, OH); Dextran T 70 was from Pharmacia. Sucrose was ultrapure grade from Schwarz/Mann.

Sample Preparation. Unless otherwise indicated, all operations were carried out at 4°. Upon removal, the tumor specimens were washed with cold 0.9% NaCl solution and placed in plastic bags. The bags were sealed and immersed in crushed ice. The tissues under ice were rapidly brought to the laboratory where they were stored at -70° for less than 1 week. Frozen tissues were weighed, pulverized in a tissue pulzzerizer (Glascol Division, Thermovac), and homogenized in 4 volumes (w/v) of 0.01 M phosphate buffer (pH 7.5)-10% glycerol-0.0015 M EDTA-0.005 M dithiothreitol-0.001 M phenylmethysulfonyl fluoride-pepstatin A (5Mg/ml), 0.01 M sodium molybdate (homogenization buffer) by means of an Ultraturrax homogenizer (Janke and Kunkel Model TP 18/2) in 4 to 6 runs of 15 sec each with 60 intervals. The homogenate was centrifuged at 105,000 x g for 60 min in a Spinco L5-75 ultracentrifuge. After centrifugation, the supernatant (cytosol) was collected and, protein concentration and steroid-binding activities were assessed as described below.

Protein Assay. Protein concentration of the cytosol fraction was estimated by measuring absorbance at 260 and 280 nm and later quantitated by the Bio-Rad protein assay based on the work of Bradford (5). Thiol groups, Tris, and EDTA do not interfere with the Bio-Rad assay.

Assay of Specific [3H]Estradiol-binding Capacity. The samples (0.2 ml) to be tested were brought to 0.25 ml with homogenization buffer containing [3H]estradiol at final concentrations of 0.4, 0.8, 1.2, 2.4, and 6 nm and incubated overnight at 4° (total binding). The nonspecific binding was measured in the presence of 3.7 uM cold 17l-estradiol. The specific binding was computed by subtracting the nonspecific from the total binding. When sufficient material was not available for Scatchard analyses, the assay was performed in triplicate only with 6 nm [3H]-estradiol in the presence and in absence of cold hormone. After incubation, separation of free hormone from macromolecule-bound estradiol was accomplished by adding 0.25 ml of dextran-coated charcoal (0.05% Dextran T 70, 0.5% charcoal in 0.01 M Tris-HCl, pH 7.5) followed by incubation for 15 min at 4°. After centrifugation at 5000 x g for 5 min, the radioactivity of 0.25 ml of supernatant was measured. For all the samples, we have calculated the average and the standard deviations of the differences of the total and nonspecific values obtained for the single determinations. We have considered positive only those with average values different from 0 in a quantity higher than ±2 S.D.

Assay of Specific [3H]Progesterone-binding Capacity. The [3H]- progesterone-binding activity was measured in the presence of 10^-11 M
Steroid Receptors in Gastrointestinal Cancers

The presence of ER and PGR. Biopsies of 79 colorectal cancers have been analyzed for current with constant power of 8 watts until the voltage reached 1.25 kV.

Sucrose Gradient Centrifugation. Sucrose gradient centrifugation was performed as previously described (30).

Flatted Electrofocusing in Granulated Gel. ER analysis by isoelectric focusing in a granulated gel was performed following the method of Radola (28) with minor modification. An LKB 2117 Multiphor apparatus was used.

Sephadex G-75 superfine (5 g) was swollen in water, filtered with a glass filter funnel, and washed with 1 liter of water; after removal of the excess water with low suction, the gel was suspended in 90 ml of 5% Ampholine, pH 5 to 8. Two strips soaked with the Ampholine solution were placed on the anodic and cathodic sides. The gel suspension was poured on a tray, and the excess water was evaporated with a light flow of air. The tray was transferred onto the cooling plate with a film of water. After sedimentation of the gel, 1 ml of supernatants was collected, and the radioactivity was measured.

RESULTS

Estrogen Receptor and PGR Distribution in Colorectal Cancer. Biopsies of 79 colorectal cancers have been analyzed for the presence of ER and PGR. The incidence of both receptors is presented in Table 1. High-affinity estrogen-binding proteins were found in 18 of 79 primary colorectal cancers (22.8%). The highest concentration of binding activity was 63.9 fmol/mg of protein. The range of ER concentration was between 9.8 and 63.9 fmol/mg of protein. PGR was found in 34 of 79 colorectal tumors (43%). The highest concentration of binding activity was 63.9 fmol/mg of protein. PGR was found in 18 of 79 primary colorectal cancers (22.8%). The highest concentration was 46.8 fmol/mg of protein. Sixty-five % of the positive samples contained more than 5 fmol of PGR/mg of protein. Only 2 samples contained less than 2 fmol. When sufficient material was available, Scatchard analyses were performed. Equilibrium dissociation constants ranged from 1.65 x 10^-10 M to 8.4 x 10^-10 M (mean ± S.D., 4.65 ± 2.65) for ER and from 2.8 x 10^-9 M to 9.1 x 10^-8 M (mean ± S.D., 5.64 ± 2.2) for PGR.

In considering the distribution of PGR relative to ER (Table 2), we find that when ER is negative 36% of tumors have PGR. When ER is positive, 66.6% of tumors also have PGR. Thirty-five of 79 specimens analyzed (44%) were from male patients, and only 3 (8.6%) were positive for both receptors (Table 3), while 24 (68.6%) were negative; 9 of 44 specimens from female patients (20.4%) were positive, and 15 (34.1%) were negative for both receptors.

Table 3 shows the distribution of receptor related to menopausal state. A woman was considered as postmenopausal when menses had ceased at least 1 year prior to biopsy.

Only 9 females were premenopausal with 6 (66%) positive for ER and PGR. Three of 35 postmenopausal patients were positive for both receptors. It appeared that premenopausal patients were more likely to have tumors positive for both receptors than postmenopausal. The frequency of PGR-positive tumors, however, was higher in postmenopausal than in premenopausal women.

Estrogen Receptor and PGR Distribution in Stomach Cancer. ER and PGR were assayed in 56 specimens of stomach adenocarcinomas. ER was found in 8 tumors (14.3%), and PGR was found in 14 tumors (25%). The highest concentration of binding activity was 120.7 fmol/mg of protein for ER and 12.6 fmol/mg of protein for PGR. The range of receptor concentration was 9.2 and 120.7 fmol/mg of protein for ER and 1.4 and 12.6 for PGR. Sixty-two % of the positive samples contained more than 5 fmol of PGR/mg of protein with only one sample containing less than 2 fmol. Scatchard analysis was performed only on 3 specimens for ER and on 2 specimens for PGR. The Kp for ER was 2.1 ± 0.7 x 10^-10 M. The Kp for PGR was 2.4 and 4.2 x 10^-6 M. The distribution of PGR relative to ER is shown in Table 2. Two of 8 samples positive for ER were also positive for PGR (25%). The patients (44 of 56) were mostly male (Table 4). Only 2 females were premenopausal, and both were PGR and ER negative.

It is worth mentioning that, when it was possible, we have assayed ER and PGR in metastatic lymph nodes. In receptor-
negative tumors, lymph nodes were also negative; while in 3 cases of primary tumors, ER negative and PGR positive, we have found the same pattern of receptor positivity in the lymph nodes with comparable concentrations of PGR (data not shown). One of our female patients had a synchronous mammary adenocarcinoma. The breast cancer in this woman was ER and PGR positive (58 and 20 fmol/mg of protein, respectively), while the stomach adenocarcinoma was only ER positive (17 fmol/mg of protein).

Characterization of ER of Stomach Adenocarcinomas. The sedimentation constant of colorectal adenocarcinoma ER has been determined using sucrose gradient centrifugation. McClendon et al. (20) found ER in the 8S sedimentation fraction.

We published (29) a sedimentation constant of 4.5S both in low and high (0.4 M KCl) salt concentration and also showed that the presence during the tissue homogenization of a serine protease inhibitor such as phenylmethylsulfonyl fluoride was required to avoid the ER sedimented in the 3S region of the gradient both in low and high ionic strength. Typical sedimentation profiles of estrogen receptors from human stomach adenocarcinomas are shown in Chart 1. The profiles shown by the closed circles represent binding in the presence of only tritiated estradiol while those presented by the triangles represent the association of labeled steroid with nonspecific sites as measured in the presence of unlabeled competitor. The profiles shown in Chart 1 are similar to those seen in target tissues and in the majority of

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### Table 3

<table>
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<tr>
<th>Receptor</th>
<th>No. of patients</th>
<th>Male</th>
<th>Female</th>
<th>Premenopausal</th>
<th>Postmenopausal</th>
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<td>ER-positive, PGR-positive</td>
<td>12</td>
<td>15.2</td>
<td>3</td>
<td>8.6</td>
<td>9</td>
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<td>7.6</td>
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<td>0</td>
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<td>27.8</td>
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<td>22.9</td>
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<td>ER-negative, PGR-negative</td>
<td>39</td>
<td>49.4</td>
<td>24</td>
<td>68.6</td>
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### Table 4

<table>
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<th>Receptor</th>
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<th>Male</th>
<th>Female</th>
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<th>Postmenopausal</th>
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<td>10</td>
<td>4</td>
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<tr>
<td>ER-negative, PGR-positive</td>
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<td>13.3</td>
<td>8</td>
<td>18.2</td>
<td>0</td>
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<tr>
<td>ER-negative, PGR-negative</td>
<td>40</td>
<td>73.3</td>
<td>30</td>
<td>68.2</td>
<td>10</td>
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Chart 1. Sedimentation rate of stomach adenocarcinoma ER on sucrose gradients. Typical sedimentation patterns on 10 to 30% sucrose gradients of 0.2-ml samples from cytosol of stomach adenocarcinomas after incubation at 4° with 6 nm [3H]estra
diol in the absence (■) or in the presence (△) of 3.7 M cold 17β-estradiol. Free estradiol was absorbed on dextran-coated charcoal before applying the sample on the gradient. Arrows, sedimentation rate of bovine plasma albumin (4.45S) used as standard reference. Specific binding (○) was obtained by subtracting nonspecific binding (△) from total binding (■). A and B, sedimentation profiles of 2 different stomach adenocarcinomas.
The association of breast and gastrointestinal cancers is now well known (1, 11, 13) and common etiological factors have been suggested, particularly of a dietary nature (9, 12, 19). Schoenberg et al. (29) reported an excess at least 50% observed over expected second primary lesions when the initial primary tumor was in the breast and the second primary tumor was either in the digestive system or the genital organs.

Herrmann (11) reported a 6.7% incidence of multiple primary malignant neoplasm in a series of 436 patients who had breast cancer and that the association with gastrointestinal cancer was more frequent than it was with cancer of the genitalia.

Recently, Alford et al. (1) and McClendon et al. (20) have found ER in 30 and 24%, respectively, in primary colon cancers analyzed. We also found (30, 31) that a significative percentage of human colon cancers contain measurable steroid hormone receptor sites. A more extensive analysis of the presence of ER and PGR in colorectal cancers has been presented in this paper. Furthermore, we have assayed these receptors also in stomach adenocarcinomas, and we found that a discrete number of these cancers are positive for at least one of these receptors.

The use of a large excess of hydrocortisone in the assay method for PGR excludes the possibility that the high positivity for this receptor may be due to the binding of progesterone to glucocorticoid receptor.

The influence of the sex on the presence of ER and PGR in colorectal and stomach adenocarcinomas is shown in Table 3 and 4, respectively. Adenocarcinoma of the stomach affects men more frequently than woman. The contrary occurs for colorectal tumors (24). In women, the positivity for at least one receptor in colon adenocarcinomas is much higher than in male patients. The contrary is observed in stomach cancer where only 16% of females are positive for at least one receptor versus 26% of positivity in male patients.

The influence of the menopausal state on the receptor distribution is also barely significant in stomach adenocarcinomas. In colorectal tumors, it seems that the presence of both receptors in the same tumor is higher in premenopausal than in postmenopausal women even if the positivity for at least one receptor has the same frequency in postmenopausal women.

The presence of steroid-binding proteins in non-target tissue tumors does not mean that we are dealing with steroid receptors. Several nonreceptor proteins binding estradiol or progesterone may be present in neoplastic tissues. For this reason, we studied some physicochemical characteristics of the estradiol- and progesterone-binding proteins found in gastrointestinal tumors, first of all the dissociation constant. In the measured specimens, the $K_d$ is in the order of the magnitude of the dissociation constant of ER and PGR of normal and neoplastic target tissues.

When the amount of tissue was sufficient, we investigated other properties of estradiol-binding proteins. Unfortunately, we had never enough neoplastic tissue to measure the same properties for progesterone-binding proteins but, as discussed above, the measured dissociation constant suggests that we are dealing with PGR.

Several laboratories use sucrose gradient method for the clinical determination of estrogen receptor principally because this technique permits the visualization of these proteins in tissue extracts as well as providing a quantitative estimate of the specific binding activity.

We used sucrose gradient method to measure sedimentation constant of this protein in stomach and colorectal cancers. In previous papers (20, 30), it has been shown that the estradiol-binding protein of colorectal adenocarcinomas sediments at 4.5S or 8S when centrifuged in a sucrose gradient and has an isoelectric point of 6.5.

In stomach adenocarcinomas, we have found that the ER sediments in 8S or in 4 to 5S regions of the gradient and that,
at least for this property, it is identical to the ER either of normal and neoplastic target organs (23) or of the other tumors (20, 30, 32) of nonclassic target organs. Furthermore, the isoelectric point of ER is equal to isoelectric point measured for ER in normal and neoplastic tissue (27, 30, 34).

The presence of ER and PGR brings out the question of the role of these hormones on the neoplastic growth of gastrointestinal cancers. With respect to colon adenocarcinomas, the higher incidence in the female sex, the reported high frequency of this cancer in patients with a history of breast tumors, the presence of specific ER and PGR, suggest that the growth of these tumors may be influenced by steroid hormones.

On the contrary, the higher incidence of stomach cancer in men than in women minimizes a role of estrogen in tumor induction. The meaning of the presence of ER and PGR in these tumors remains intriguing, and further investigations are required to answer the question if some stomach cancer may also be endocrine dependent or if the presence of these proteins is due to a causal perturbation of the regulation of gene expression. Nevertheless, we cannot exclude that the presence of these receptors in a significant percentage of gastrointestinal tumors can represent a new property of these neoplastic cells that may now have acquired a steroid hormone dependence of the growth. This suggests the hypothesis that, besides chemotherapy and radiotherapy, hormonal manipulation may also result useful in receptor-positive gastrointestinal tumors.

To this purpose, it is worth mentioning that positive results have recently been obtained in a group of patients with nonoperable cardias tumors treated with polychemotherapy and medroxyprogesterone acetate as compared to another group of patients treated only with polychemotherapy (7, 8).

REFERENCES


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