Human Renal Cell Carcinoma as a Hormone-dependent Tumor

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Abstract

On the basis of experimentally induced renal cancer and clinical, statistical, and epidemiological observations, it has been hypothesized that human renal cancer is hormone dependent. Hormone therapy for this neoplasm has been proposed.

Recent knowledge of the mechanism of action of steroid hormones and demonstration of steroid receptors in the kidney of various experimental animals led us to investigate normal human kidney and human renal cell carcinoma for the presence of steroid receptors.

By means of agar gel electrophoresis and of protamine sulfate assay techniques, estradiol receptors (ER) and progesterone receptors (PR) were sought in the cytosol fraction of renal cancer in 23 patients. ER was found in 61% and PR in 61% of the carcinomas studied: 39% of the tumors were positive for both ER and PR; 17% were negative for both ER and PR. ER and PR were also examined in the nuclear fraction of three renal cancers: estradiol receptor was found in two and progesterone receptor in three of the three tumors examined. These findings were considered strongly supportive of the hypothesis of the hormone dependence of human renal cancer.

Progestational therapy, commenced after nephrectomy in 18 of the 23 patients investigated, was considered a useful adjuvant in 14 cases; 3 of 4 patients with receptor negative cancers did not respond favorably to progestins, while one is still alive more than 33 months after surgery. One patient with ER-positive and PR-negative tumor, given antiestrogenic therapy following nephrectomy, died after 8 months of treatment. The follow-up of the remaining patients is reported.

Introduction

Renal cell carcinomas have been induced with female sex hormones in male mice and in female guinea pigs (31), as well as in the male Syrian hamsters and in female animals treated after ovariectomy or at the time of low progesterone secretion or before reproductive maturity (19, 23). Endocrine balance following resection of ovaries has been seen to delay occurrence of tumors in various organs, including kidney (3). The growth of these hormone-dependent tumors is inhibited by certain steroidal estrogen antagonists such as testosterone propionate, progesterone, and deoxycorticosterone (19, 32).

Some clinical observations such as the significant sex difference of renal cell carcinoma (twice as common in men as in women) and its variation with the cessation of gonadal activity, the racial differences, and the regression of metastatic renal cancer during administration of progestin or androgen (greater in men than in women) led to the hypothesis that human renal cancer is a hormone-dependent tumor (2).

Bloom (1) used MPA in the treatment of inoperable renal carcinoma and observed a 16% response rate. Bracci and Di Silverio (5) reported a higher incidence of metastases in nephrectomized patients compared to nephrectomized patients treated with high doses of progestational compounds.

The presence of steroid receptors has recently been reported by several authors (8, 18, 20, 21, 30) in the kidneys of experimental animals and in normal human kidney, as well as in human renal cell carcinoma (4, 10, 12, 15).

On this basis studies have been performed on ER and PR of human renal cell carcinoma in patients treated after nephrectomy with progestins to establish whether the different responses to progestational therapy could be related to the presence of steroid receptors.

Materials and Methods

Chemicals and Reagents. 17β-[2,4,6,7-3H]Estradiol (105 Ci/mmol) was purchased from New England Nuclear, Frankfurt-Main, Germany; unlabeled 17β-estradiol was provided by Vister, Como, Italy. Tritiated R5020 (51 Ci/mmol) and unlabeled R5020 were kindly supplied by Dr. J. P. Raynaud, Roussel-Uclaf, Romainville, France. The radiocchemical purity of labeled compounds was determined either by paper or thin-layer chromatography. Reagents used were: sodium phosphate monobasic, sodium phosphate dibasic, glycerol, and sodium acetate from Carlo Erba, Milan, Italy; monothioglycerol from Calbiochem, San Diego, Calif.; charcoal from Riedel-De Haen, Seelze-Hannover, Germany; dextran from Schuchardt, Munich, Germany; naphthalene, POPOP, dimethyl-POPOP, sodium diethylbarbiturate, dioxane, toluene, methanol, and HCl from Merck, Darmstadt, Germany; agar purum from Behringwerke, Marburg-Lahn, Germany; protamine sulfate from Serva Feinbiochemica, Heidelberg, Germany.

Sample Preparations. Renal tumors were immediately processed or stored at –22°C. The processed specimens were thinly sliced and preincubated with 20 nM cold 17β-estradiol for 5 hr at 25°C or with 20 nM cold progesterone for 1 hr at 20°C. The slices were then rinsed with cold NaCl.
solution, minced and homogenized in 2 volumes of phosphate buffer (5 mM sodium phosphate, 10% glycerol, and 1 mM monothioglycerol, pH 7.5). For cytosol preparation the homogenate was centrifuged at 10,000 × g for 30 min at 2°, and the supernatant was centrifuged again at 200,000 × g for 90 min at 2° (10). For nuclear preparation tissue was homogenized in 3 volumes of phosphate buffer, the homogenate was centrifuged at 800 × g for 10 min at 2°, and the precipitate was washed 3 times with cold phosphate buffer. Salt extraction was performed by adding 0.4 M KCl in phosphate buffer to the precipitate, which was allowed to stand on ice for 30 min. Nuclear extract was then centrifuged at 800 × g for 10 min and then at 100,000 × g for 60 min.

Incubation Experiments. When tissues were processed after storage, incubation of cytosol fractions with tritiated estradiol or tritiated R5020 in the presence and in the absence of a 500-fold excess of equivalent cold steroids was carried out for 18 hr at 4°. Other details on incubation procedures and the agar gel electrophoresis at high voltage and low temperature (34) have been already reported (12). Some of the cytosol preparations, as well as nuclear fractions, were analyzed with the exchange assay. Therefore, cytosol incubation was performed with 20 nM tritiated estradiol in the presence and in the absence of a 100-fold excess of cold estradiol for 5 hr at 25°, and with 20 nM tritiated R5020 in the presence and in the absence of a 100-fold excess of cold R5020 for 1 hr at 20°. Nuclear incubation time was 3 hr at 30° and 30 min at 25°, respectively, for tritiated estradiol and for tritiated R5020 with and without the addition of the equivalent cold steroid.

Protamine sulfate assays were performed with 0.2 ml of cytosol and 0.4 ml of nuclear fraction diluted 1:4 as described by Zava and McGuire (36). Aliquots of different fractions were taken for protein and DNA determinations (9, 22).

Radioactivity was measured with a Packard Tri-Carb Model 3380 β-scintillation counter by adding 10 ml of Bray’s (6) solution to the samples or to the frozen agar gel fragments.

Clinical Studies. Investigations were carried out in 23 patients (18 males and 5 females), with a mean age of 44 years, 20 of whom (87%) showed no radiological evidence of metastases at the time of nephrectomy, while 3 patients (13%) presented lung metastases. One patient was not treated; 3 patients were treated with combined radiotherapy and chemotherapy; one patient was treated with tamoxifen, 10 mg p.o., twice a day; 18 patients were treated with MPA, 250 mg/day i.m. for 1 month, every other day for another month, then weekly until the end of the third month after nephrectomy, then every 10 days until the 12th month, and thereafter every 20 days for at least 2 to 3 years after surgery.

Results

As in normal human kidney cytosol (4, 10, 29), both estradiol and progesterone receptors have been found in the cytosol of human renal cell carcinoma. Although the results of quantitative experiments are not reported in this paper, a lower binding capacity of human renal cancer cytosol with respect to that of normal kidney cytosol was found, perhaps due to a nuclear translocation of the receptors in the neoplastic tissue and to a greater nuclear uptake as demonstrated by Fanestil et al. (15).

The distribution of estradiol and progesterone cytosol receptors in the human renal cell carcinomas studied is presented in Table 1. Estradiol receptor was found in 14 tumors (61%), and progesterone receptor was found in 14 tumors (61%); 39% of the tumors were positive for both ER and PR and 17% (4 of 23 cancers) were negative for both ER and PR.

The presence of estradiol and progesterone receptors in the nuclear fraction of human renal cancer is reported in Table 2; only 3 cancers have been examined thus far. The comparison between cytoplasmic and nuclear receptors shows a greater nuclear concentration of both ER and PR. Since the exchange assay technique was used, data are representative of total receptor sites.

As reported in Table 3, the patients were divided into 4 groups according to receptors studied: Group 1, both ER- and PR-positive tumor (ER- PR+); Group 2, ER-positive and PR-negative tumor (ER+ PR-); Group 3, ER-negative and PR-positive tumor (ER- PR+); and Group 4, both ER- and PR-negative tumor (ER- PR-).

Evaluation of response in metastatic disease can be based upon the following criteria: for complete remission, disappearance of all demonstrable lesions and normalization of all abnormal laboratory values; for partial remission, a greater than 50% decrease in the sum of the products of diameters of all measurable lesions; for objective response, tumor shrinkage of less than 50%; for stabilization, tumor size unchanged following at least 2 months of therapy; for progression, a greater than 25% increase in the sum of the products of diameters of any measurable lesion (16).

Only 3 (13%) of the 23 patients examined had a metastatic disease and they died a few months after surgery.

Table 1

<table>
<thead>
<tr>
<th>ER</th>
<th>PR</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER+</td>
<td>9 (39)</td>
<td>5 (22)</td>
</tr>
<tr>
<td>ER-</td>
<td>5 (22)</td>
<td>4 (17)</td>
</tr>
<tr>
<td>14 (61)</td>
<td>9 (39)</td>
<td>23</td>
</tr>
</tbody>
</table>

* Numbers in parentheses, percentage.

Table 2

<table>
<thead>
<tr>
<th>Patient</th>
<th>ER+</th>
<th>ER-</th>
<th>PR+</th>
<th>PR-</th>
</tr>
</thead>
<tbody>
<tr>
<td>N. L.</td>
<td>0.12</td>
<td>0.64</td>
<td>0.94</td>
<td></td>
</tr>
<tr>
<td>P. G.</td>
<td>0.59</td>
<td>1.15</td>
<td>0.13</td>
<td>2.20</td>
</tr>
<tr>
<td>R. F.</td>
<td>0.11</td>
<td>2.48</td>
<td>0.15</td>
<td>0.20</td>
</tr>
</tbody>
</table>

* Rc, cytosol receptor; Rn, nuclear receptor.
Table 3
Clinical follow-up of the patients examined and ER and PR receptors in the renal cell carcinoma

<table>
<thead>
<tr>
<th>Patients</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>TNM</th>
<th>Treatment</th>
<th>Mos. of control</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER+ PR−</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N. L.</td>
<td>66</td>
<td>M</td>
<td>T₄N₁M₀</td>
<td>Radiochemotherapy</td>
<td>8-Exitus</td>
</tr>
<tr>
<td>S. M.</td>
<td>50</td>
<td>M</td>
<td>T₄N₁M₀</td>
<td>Radiochemotherapy</td>
<td>4-Exitus</td>
</tr>
<tr>
<td>F. R.</td>
<td>58</td>
<td>M</td>
<td>T₄N₁M₀</td>
<td>Radiochemotherapy</td>
<td>2-Exitus</td>
</tr>
<tr>
<td>S. G.</td>
<td>48</td>
<td>M</td>
<td>T₄N₁M₀</td>
<td>MPA</td>
<td>35</td>
</tr>
<tr>
<td>B. L.</td>
<td>62</td>
<td>F</td>
<td>T₄N₁M₀</td>
<td>MPA</td>
<td>8</td>
</tr>
<tr>
<td>P. A.</td>
<td>62</td>
<td>F</td>
<td>T₄N₁M₀</td>
<td>MPA</td>
<td>18</td>
</tr>
<tr>
<td>V. A.</td>
<td>64</td>
<td>M</td>
<td>T₄N₁M₀</td>
<td>MPA</td>
<td>13</td>
</tr>
<tr>
<td>R. F.</td>
<td>51</td>
<td>M</td>
<td>T₄N₁M₀</td>
<td>MPA</td>
<td>13</td>
</tr>
<tr>
<td>P. G.</td>
<td>56</td>
<td>M</td>
<td>T₄N₁M₀</td>
<td>MPA</td>
<td></td>
</tr>
<tr>
<td>ER+ PR−</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>U. G.</td>
<td>50</td>
<td>M</td>
<td>T₄N₁M₀</td>
<td>Antiestrogen</td>
<td>8-Exitus</td>
</tr>
<tr>
<td>F. A.</td>
<td>49</td>
<td>M</td>
<td>T₄N₁M₀</td>
<td>MPA</td>
<td>12</td>
</tr>
<tr>
<td>V. A.</td>
<td>60</td>
<td>M</td>
<td>T₄N₁M₀</td>
<td>MPA</td>
<td>12</td>
</tr>
<tr>
<td>S. N.</td>
<td>57</td>
<td>F</td>
<td>T₄N₁M₀</td>
<td>MPA</td>
<td>12</td>
</tr>
<tr>
<td>A. L.</td>
<td>65</td>
<td>M</td>
<td>T₄N₁M₀</td>
<td>MPA</td>
<td>13</td>
</tr>
<tr>
<td>ER− PR+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D. A. F.</td>
<td>60</td>
<td>F</td>
<td>T₄N₁M₀</td>
<td>MPA</td>
<td>4-Exitus</td>
</tr>
<tr>
<td>C. A.</td>
<td>50</td>
<td>F</td>
<td>T₄N₁M₀</td>
<td>No treatment</td>
<td>4-Exitus</td>
</tr>
<tr>
<td>Z. G.</td>
<td>45</td>
<td>M</td>
<td>T₄N₁M₀</td>
<td>MPA</td>
<td>36</td>
</tr>
<tr>
<td>G. F.</td>
<td>60</td>
<td>M</td>
<td>T₄N₁M₀</td>
<td>MPA</td>
<td>18</td>
</tr>
<tr>
<td>V. R.</td>
<td>52</td>
<td>M</td>
<td>T₄N₁M₀</td>
<td>MPA</td>
<td>22</td>
</tr>
<tr>
<td>ER− PR−</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. E.</td>
<td>76</td>
<td>M</td>
<td>T₄N₁M₀</td>
<td>MPA</td>
<td>9-Exitus</td>
</tr>
<tr>
<td>G. G.</td>
<td>60</td>
<td>M</td>
<td>T₄N₁M₀</td>
<td>MPA</td>
<td>5-Exitus</td>
</tr>
<tr>
<td>C. O.</td>
<td>53</td>
<td>M</td>
<td>T₄N₁M₀</td>
<td>MPA</td>
<td>33</td>
</tr>
<tr>
<td>P. A.</td>
<td>74</td>
<td>M</td>
<td>T₄N₁M₀</td>
<td>MPA</td>
<td>8-Exitus</td>
</tr>
</tbody>
</table>

a Tumor-nodes-metastasis classification (Unio International Contre Cancrum, Geneva 1974).
b Tamoxifen.

eone of these patients (ER− PR−, Patient S. E.), treated with pharmacological doses of MPA soon after nephrectomy, presented unchanged size of lung metastasis for 8 months, we are not inclined to consider this result as stabilization of the disease. In fact, full metastatic disease and death occurred 1 month later, i.e., 9 months after nephrectomy. The 2 other patients with metastases, one treated with antiestrogen (ER+ PR−, Patient U. G.) and the other not given any drug (ER− PR+, Patient C. A.), died 8 and 4 months, respectively, after nephrectomy.

In 18 of the 20 patients treated with MPA who did not present any metastatic lesion at time of nephrectomy, the parameters studied at follow-up were primarily normalization of all laboratory findings and “time-free” interval (i.e., the time elapsing before the appearance of metastases). As is, in fact, well known, patients with renal cell carcinoma usually develop metastases 18 to 24 months after nephrectomy (2, 5, 27). Only 4 of these 18 patients have exceeded that limit and are still alive without any objective or subjective sign of disease 35, 33, 36 and 33 months, respectively, after surgery. One of these patients (Patient C. O.) had a receptor-negative tumor; the other 3 belong to the groups that we consider to have a hormone-dependent tumor (ER+ PR+, Patients S. G., B. L.; ER− PR+, Patient Z. G.). Three patients, one in the first group (Patient P. A.) and two in the third group (Patients C. F. and V. R.), can be considered good responders to progestin treatment since they did not develop metastases within 18 to 22 months of nephrectomy.

The 7 patients [V. A., R. F., and P. G. in the first group (ER+ PR+) and F. A., V. A., S. N., and A. L. in the second group (ER+ PR−)], who did not develop metastases had too short a follow-up to allow any conclusion to be drawn. Nevertheless, stabilization of the disease can be hypothesized for these patients.

Of the 23 patients studied, 9 died a few months after surgery; as already mentioned, 3 of these patients (13%) had a metastatic disease. Of the remaining 6 (26%), 3 patients (Patients N. L., S. M., and F. R.) with ER+ PR+ tumors, who did not receive hormonal therapy, died a few months after nephrectomy, radiotherapy and chemotherapy being useless; 2 patients (Patients G. G. and P. A.) had ER− PR− tumors; one patient (Patient D. A. F.) with an ER− PR+ tumor died a few months after nephrectomy while under MPA treatment.

Discussion
In a series of 176 patients observed between 1967 and 1976 with renal cell carcinoma with or without metastases, Bracci and Di Silverio (5) reported a significant difference between nephrectomized patients and those nephrectomized and treated with high dose of progestational compounds. Metastases, in fact, developed in 25% of nephrectomized patients and in 8% of 124 nephrectomized and progestin-treated patients. Metastases appeared during the administration of progestins in 7 patients and after...
withdrawal of treatment in 3 patients, leading to the hypothesis that development of metastases was due to the lack of inhibition of the tumor growth by progestins (14).

While a large amount of data reported in the literature confirm that hormone treatment may be the treatment of choice in the management of metastatic renal cell carcinoma (2, 7, 27), according to other authors (13, 26, 33) no objective response is obtained after either progestagens and/or androgens (17, 24, 28, 35), and the remission rate of 16 to 20% in cases of metastatic hypernephroma with gestational agents is considered due to spontaneous regression of this tumor. Montie et al. (25), however, reported on the incidence of regression of metastases following nephrectomy in 9 series and found 4 cases of regression in 474 patients collected (0.8%).

The aim of the present work was to correlate the various responses to progestational therapy with the presence of renal cancer steroid receptors. The identification, in fact, of ER and PR in the cytosol and in the nuclear fraction of kidney tumor led us to hypothesize that, like human breast cancer which is unresponsive to hormonal therapy in the absence of female steroid receptors, progestational therapy may be useful in the treatment of renal cell carcinoma in which both ER and PR are present (11). It is tempting to suggest that the presence of receptors may strongly support the hypothesis of the hormone dependence of human renal cell carcinoma, which could therefore be susceptible to hormone treatment.

Partial remission or stabilization was obtained in 14 of 18 patients nephrectomized and treated with MPA. In fact, 6 of the 9 patients with estradiol- and progesterone-positive cytosol receptors (ER+ PR+), treated with MPA, are still alive and did not develop metastases which, in untreated patients, usually occur within 18 to 24 months of nephrectomy (2, 5, 27); 3 of these patients, treated with combined radiotherapy and chemotherapy, died in a few months after surgery.

Transient hormone dependence was postulated for the 5 patients characterized by estradiol-positive and progesterone-negative cytosol receptors (ER+ PR−) and for the 5 patients with estradiol-negative and progesterone-positive (ER− PR+) cancers. Of the patients with ER+ PR− cancer, treated with MPA, 4 are alive 1 year after surgery. Three of the 4 patients with ER− PR+ cancer, treated with MPA, are alive after 36, 18, and 22 months without any sign of disease; only one patient died unexpectedly 4 months after nephrectomy. One of the 4 patients with ER− PR− cancer is still alive after 33 months, but 3 patients died in a few months although MPA treatment was commenced soon after surgery.

Therefore, receptor studies may provide some useful information in establishing the treatment of human renal carcinoma. Progestational therapy could be the treatment of choice in hormone-dependent renal cancer characterized by ER and PR (ER+ PR+) as well as in transient hormone-dependent renal cancer characterized by ER or PR only (ER+ PR−, ER− PR+). When the tumor has lost its hormone dependence (ER− PR−), patients can only be treated with chemotherapy, associated with radiotherapy and immunotherapy.

References


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NOVEMBER 1978

4343
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