Cancer Chemother Pharmacol (1981) 6: 151–154

Cancer Chemotherapy and Pharmacology



# Hormone Receptors in Renal Cell Carcinoma

## Their Utility as Predictors of Response to Endocrine Therapy

John Pearson[1], Michael A. Friedman[2], and Philip G. Hoffman, Jr[3]

[1] Department of Urology
[2] The Cancer Research Institute, 1282 Moffitt
[3] Department of Obstetrics, Gynecology, and Reproductive Sciences
The University of California, San Francisco, California 94143, USA

***Summary.*** *We have performed 23 estrogen and 24 progesterone receptor assays on tumor specimens from 26 patients with renal cancer, ten of whom subsequently received hormonal therapy for metastatic disease. Only one of these specimens contained measurable estrogen receptor levels, three contained low, but measurable progesterone receptor levels, and the remaining specimens contained no measurable estrogen or progesterone receptors. None of ten patients with metastases showed evidence of an objective response to hormonal therapy. Our data suggest that few, if any, renal cancers have high titers of estrogen or progesterone receptors, and that those patients whose tumors have low receptor titers are unlikely to respond to hormonal therapy.*

## Introduction

The prognosis for patients with advanced or metastatic renal cell carcinoma is poor. Neither radiotherapy nor treatment with cytotoxic drugs has increased objective responses or prolonged survival. Hormonal manipulation has long been advocated as therapy for these patients. Endocrine dependence of renal cancer in humans was suggested by experiments with estrogen-induced renal adenocarcinoma in the male golden hamster. In these animals, estrogens promoted while testosterone and progesterone markedly inhibited the production or growth of renal adenocarcinomas [10]. Moreover, high levels of both estrogen and progesterone receptors have been demonstrated in the cytosol of these hamster tumors [11, 12].

In contrast, only a limited number of renal cancer patients have responded to hormonal therapy [1, 2, 14], and it would be desirable to be able to predict which patients would be most likely to respond. In human breast cancer, the presence of hormone receptors is predictive of objective response to endocrine therapy [9, 13]. To test whether a similar clinically useful role for receptors can be developed for renal carcinoma, we have measured estrogen and progesterone receptors in patients with renal adenocarcinoma, and have attempted to correlate these results with the responses of those patients with measurable disease to hormonal therapy.

*Reprint requests should be addressed to* M. A. Friedman

## Materials and Methods

Sephadex G-25 (medium grade), Tris-HCl and EDTA (both reagent grade), and cortisol and dihydrotestosterone (DHT) were purchased from Sigma Chemical Co., St. Louis, MO, USA. Dithiothreitol was obtained from Calbiochem, San Diego, CA. The source of glycerol (spectrometric grade) was Mallingkrodt Chemical Works, St. Louis, MO, USA, Charcoal (Norit A) was obtained from J. T. Baker Chemical Co., Phillipsburg, NJ, USA and dextran (Dextran radioimmunoassay) was obtained from Schwarz/Mann, Orangeburg, NY, USA (2,4,6,7-$^3$H)-estradiol and (2,4,6,7-$^3$H)-progesterone were purchased from New England Nuclear, Boston, MA, USA.

[$^3$H]-Estradiol and [$^3$H]-progesterone were repurified by Celite column partition chromatography every 2 weeks, according to the method of Siiteri [17]. The solvent system used for both radiolabeled steroids was isooctane/ethylene glycol.

Specimens of human renal adenocarcinoma were collected from 26 patients, and frozen in liquid nitrogen within 15 min. Samples were stored in liquid nitrogen until assayed, usually within 1 week of collection. The tissue was powdered in a Thermovac Autopulverizer stainless steel mortar cooled to −90° C. The powder was weighed and added to ice-cold TEDG buffer (0.01 *M* Tris-HCl, 0.0015 *M* EDTA, 0.5 m*M* dithiothreitol, and 10% glycerol), pH 7.4, at 24° C. The final concentration was 100 mg tissue per ml.

The pulverized tissue was homogenized three times for 10 s each time, with alternate 1-min cooling periods, in a Polytron tissue homogenizer with a rheostat setting of 5. Cytosol was prepared from the homogenate by centrifugation at 50,000 *g* for 90 min. The

0344-5704/81/0006/0151/$ 01.00

soluble protein concentration was 3–5 mg/ml, as determined by the Coomassie Blue protein assay of Bradford [5].

[$^3$H]-Estradiol dissolved in absolute alcohol, together with a 200-fold molar excess of nonradioactive dihydrotestosterone to prevent binding of [$^3$H]-estradiol to sex binding globulin (SBG), was added to 12 × 75 mm disposable test tubes and evaporated to dryness under a stream of nitrogen. Seven concentrations of radioactive ligand, from 0.1–10 n*M*, were used for saturation analysis. Cytosol (0.2 ml) was added and the tubes were vortexed and incubated for 2 h at 4° C.

After incubation, an aliquot (0.05 ml) was removed to determine the total amount of radioactive ligand present in each incubation tube. The amount of ligand bound in each tube was determined by a Sephadex gel filtration method as follows: Sephadex was swollen for at least 24 h at 4° C in TE buffer (0.01 *M* Tris HCl/0.0015 *M* EDTA). Columns were fashioned from 5-in. Pasteur pipettes, gently plugged with glass filter disks (Gelman – 61630, type A-E) cut to 3/16 in. diameter with a paper punch. Columns were packed with Sephadex, with a 1-ml void at the top. The columns were stored in TE buffer at 4° C until ready for use.

During the assay, the Sephadex columns were maintained at 0–1° C. Samples (0.1 ml) from each incubation were applied to individual columns. After 60 s to allow the sample to enter the Sephadex, the sample was eluted into scintillation vials with 0.75 ml TEDG buffer, and the amount of bound radioactive ligand in the eluent was determined.

Progesterone receptor titers and dissociation constants were determined according to the method of Seematter and co-workers [16]. [$^3$H]-progesterone was used as ligand. Binding to cortisol-binding globulin was prevented by adding 100-fold molar excess of cortisol. Ten concentrations of radioactive ligand, from 0.5–30 n*M*, were used for saturation analysis. Cytosol (0.2 ml) was added and the tubes were vortexed and incubated at 4° C for 3 h.

After removal of a 0.05-ml aliquot to determine the total amount of radioactive ligand present, the bound ligand was separated by the dextran-coated charcoal method. After 12 min incubation at 4° C, the tubes were centrifuged at 800 *g* for 20 min, and 0.1 ml supernatant was removed for counting. The efficiency of the charcoal extraction remained 98% up to the addition of a 500-fold excess of cold ligand [17].

**Table 1.** Patient characteristics

| Patient no. | Sex | Age | E2 | | Progesterone | | Hormone Rx post-op. | Tumor regression |
|---|---|---|---|---|---|---|---|---|
| | | | Titer (fm/mg) | Kd ($\times 10^{-9}$) | Titer (fm/gm) | Kd ($\times 10^{-9}$) | | |
| 1 | M | 56 | 0 | – | 0 | – | Provera 100 mg PO tid | None |
| 2 | M | 54 | 0 | – | Not done | – | Provera 100 mg PO tid | None |
| 3 | M | 50 | 0[a] | – | Not done | – | Provera 500 mg/week | None |
| 4 | M | 51 | 0 | – | 210 ± 190 | 0.1 | No | |
| 5 | M | 62 | 25 ± 12 | 0.01 | 0[a] | – | Depoprovera 1 g/week + Halotestin 50 mg Then: Tamoxifen | None |
| 6 | M | 55 | 0 | – | 0[a] | – | No | |
| 7 | M | 54 | 42 ± 33 | 0.01 | 133 ± 78 | 0.1 | Halotestin 40 mg PO daily | None |
| 8 | M | 46 | 74 ± 35 | 0.01 | 0[a] | – | Provera 400 mg 1*M* 2×/week | None |
| 9 | M | 82 | 196 ± 178 | 0.01 | 0[a] | – | No | None |
| 10 | F | 55 | 0 | – | 189 ± 56 | 0.1 | No | None |
| 11 | M | 36 | 0 | – | 0[a] | – | No | |
| 12 | M | 67 | 0 | – | 0[b] | – | Megace 40 mg daily | No evaluable disease (humerus) |
| 13 | M | 81 | 123 ± 115 | 0.19 | 564 ± 87 | 0.11 | Depoprovera 1 g/week | None |
| 14 | M | 72 | 0 | – | 0[b] | – | Megace 40 daily + CCNU + Velban | None |
| 15 | M | 60 | 0 | – | 0[b] | – | No | |
| 16 | F | 84 | 0 | – | 0 | – | No | |
| 17 | M | 60 | 162 ± 139 | 0.24 | 126 ± 71 | 0.1 | No | |
| 18 | M | 62 | 182 ± 36 | 0.24 | 484 ± 87 | 0.1 | No | |
| 19 | M | 53 | 0 | – | 0[a] | – | Provera 100 mg PO tid | None |
| 20 | M | 50 | – | – | 0[b] | – | No | |
| 21 | F | 52 | 0 | – | 0[c] | – | No | |
| 22 | M | 38 | 103 ± 38 | – | 206 ± 537 | – | No | |
| 23 | M | 62 | 0 | – | 0[a] | – | No | |
| 24 | M | 58 | – | – | 321 ± 138 | 0.1 | Provera 1 g/week | Progression |
| 25 | M | 55 | – | – | 0 | – | No | |
| 26 | F | 52 | 42 ± 14 | – | 489 ± 560 | – | No | |

[a] < 500 fmole/mg tissue (± 95% confidence limits);
[b] < 300 fmole/mg tissue (± 95% confidence limits);
[c] No evidence of receptor. Data inadequate to calculate confidence limits
No notation, < 100 fmole/mg tissue (± 95% confidence limits)

Binding capacity and affinity were calculated by least-square fit to a three-parameter model [15], with reference to the algorithm described by Hoffman and co-workers [8].

Clinical data were obtained retrospectively by review of physician and hospital records and pathology reports. The stage of cancer at the time tissue was assayed for hormone receptors as well as the operations performed were noted. Hormonal therapy, both before (patient 5) and after collection of tissue, and the patient's response to this therapy were recorded. Responses to hormonal therapy administered after tissue removal were correlated with hormone receptor titers retrospectively. Receptor results were not known by the attending physicians.

## Results

Data on patients included in this study are tabulated in Table 1. Of the 26 patients, 22 were male and four female, a higher proportion than the usually reported ratio of 2–4: 1. The median age was 55 years, with a range of 36–84 years. All patients had a radical nephrectomy except one who had a partial nephrectomy in a solitary kidney, the contralateral kidney having been removed for renal adenocarcinoma 13 years earlier.

Of 23 tumors assayed for ER, only one had a significant receptor level (182 ± 36 fmol/g tissue). Of 24 tumors assayed for PR, three had significant titers (564 ± 87, 484 ± 87, 321 ± 138 fmol/g tissue). In 11 tumors assayed, there was 32.4 ± 13.3 (SD) mg cytosol protein per mg wet tumor tissue. Thus the ER-positive tumor had a titer of approximately 6 fmol/mg cytosol protein, while the highest PR titer was approximately 17 fmol/mg cytosol protein. Only one patient (no. 5) had unsuccessful hormonal therapy prior to tissue sampling for ER and PR, and this prior therapy could have diminished receptor titers.

Ten patients who developed measurable metastatic disease subsequently were treated with hormonal therapy. In all but one, therapy consisted of high-dose progestational therapy administered either orally (PO) or intramuscularly (IM). None showed any evidence of tumor regression during or after therapy.

## Discussion

Our data indicate that few tumors have significant progesterone receptor titers, which is in agreement with both the data of Bojar et al. [4] and the later data of Concolino et al., obtained with R5020 [6, 7]. Our data also indicate that few tumors have significant estrogen receptor titers. Bojar's [3] and Concolino's groups [6, 7] found estrogen receptors in human renal carcinoma, but both noted they were present in titers lower than in normal kidney.

We found that the few renal cell carcinomas that have detectable hormone receptors have them at titers near or lower than what would be considered 'positive' for breast carcinoma (≥ 250 fmol/gm tissue for ER, ≥ 500 fmol/gm tissue for PR).

To determine the value of hormone receptor assays in human renal cell carcinoma, they should be correlated with response to endocrine therapy only in patients with measurable metastatic disease so that objective criteria of response can be employed (as has been done in breast carcinoma). We assumed that there was consistency between the receptor content of the primary tumor and the metastatic deposit. By following these strict criteria, we have found no responses to progestin therapy among those tumors with low receptor titers. This is the principal value of this report, since it is the largest series of receptor measurements correlated with hormonal therapy in patients with metastatic disease. Concolino et al. [6, 7] attempted to correlate the presence of receptors with clinical course in patients who have no evidence of disease, but lacked appropriate controls. We recognize, however, that our current study was of insufficient size to permit concrete conclusions, and further studies are ongoing. A larger series of patients, containing those who have receptor-positive tumors and are receiving hormone therapy, would be an ideal study sample.

There is general agreement that the cytosol of most renal adenocarcinomas has low titers of estrogen and progesterone receptors compared with both normal renal tissue and other hormone-responsive tumors, such as breast carcinoma. Further correlation with response rates in patients with measurable disease is needed, as is evaluation of hormone receptors in nuclear material. Hamster tumors have very high titers of progesterone receptors, 13–17 times higher than normal hamster kidney [11, 12], which correlates with their extreme hormone dependence. Few (if any) human renal tumors have such high titers of progesterone or estrogen receptors. It may be that human renal carcinoma is not comparable to hamster tumors, and that this is an explanation of why most patients do not respond to hormonal therapy.

## References

1. Alberto P, Senn HJ (1974) Hormonal therapy of renal carcinoma alone and in association with cytostatic drugs. Cancer 33: 1226–1229
2. Bloom HJG (1973) Hormone-induced and spontaneous regression of metastatic renal cancer. Cancer 32: 1066–1071
3. Bojar H, Wittliff JL, Dreyfürst R, Boerminghauss F, Staib W (1975) Properties of specific estrogen-binding components in

human kidney and renal carcinoma. (Abstract) Acta Endocrinol [Suppl] (Kbh) 193: 51
4. Bojar H, Maar K, Staib W (1979) The endocrine background of human renal cell carcinoma. I. Binding of the highly potent progestin R 5020 by tumour cytosol. Urol Int 34: 302–311
5. Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72: 248–254
6. Concolino G, Marocchi A, Conti C, Tenaglia R, DiSilverio F, Bracci U (1978) Human renal cell carcinoma as a hormone-dependent tumor. Cancer Res 38: 4340–4344
7. Concolino G, DiSilverio F, Marocchi A, Bracci U (1979) Renal cancer steroid receptors: Biochemical basis for endocrine therapy. Eur Urol 5: 90–93
8. Hoffman PG, Jones LA, Kuhn RW, Sitteri PK (1980) Progesterone receptors: Saturation analysis by a solid phase hydroxyapatite absorption technique. Cancer 46: 2801–2804
9. Jensen EV, Polley TZ, Smith S, Block GE, Ferguson DJ, DeSombre ER (1975) Prediction of hormone dependency in human breast cancer. In: McGuire WL, Carbone PP, Volmer EP (eds) Estrogen receptors in human breast cancer. Raven Press, New York, pp 37–56
10. Kirkman H (1959) Estrogen-induced tumors of the kidney. III. Growth characteristics in the Syrian hamster. Natl Cancer Inst Monogr 1: 1–37
11. Li JJ, Talley DJ, Li SA, Vilee CA (1976) Receptor characteristics of specific estrogen binding in the renal adenocarcinoma of the golden hamster. Cancer Res 36: 1127–1132
12. Li SA, Li JJ, Vilee CA (1977) Significance of the progesterone receptor in the estrogen-induced and -dependent renal tumor of the Syrian golden hamster. Ann NY Acad Sci 286: 369–383
13. McGuire WL (1975) Current status of estrogen receptors in human breast cancer. Cancer 36: 638–644
14. Morales A, Kiruluta G, Lott S (1975) Hormones in the treatment of metastatic renal cancer. J Urol 114: 692–693
15. Rodbard D, Feldman HA (1975) Theory of protein-ligand interaction. Methods Enzymol 36: 3–16
16. Seematter RJ, Hoffman PG, Kuhn RW, Lockwood LC, Siiteri PK (1978) Comparison of $^{3}H$ progesterone and 6,7-$^{3}H$-17, 21-dimethyl-19-norpregna-4, 9-diene-3, 20-dione for the measurement of progesterone receptors in human malignant tissue. Cancer Res 38: 2800–2806
17. Siiteri PK (1963) The isolation of urinary estrogens and determination of their specific activities following the administration of radioactive precursors to humans. Steroids 2: 687–712

Received March 2/Accepted May 11, 1981