Suramin-Induced Decrease in Prostate-Specific Antigen Expression With No Effect on Tumor Growth in the LNCaP Model of Human Prostate Cancer

George N. Thalmann, Robert A. Sikes, Shi-Ming Chang, Dennis A. Johnston, Andrew C. von Eschenbach, Leland W. K. Chung*

**Background:** Suramin, a polysulfonated naphthylurea and a recognized antitrypanosomal agent, has shown some promise in phase II clinical trials in the management of hormone-refractory human prostate cancer. Reduction of serum prostate-specific antigen (PSA) levels has been proposed as an end point for evaluating the antitumor efficacy of treatments for hormone-refractory prostate cancer. **Purpose:** We examined the antitumor effect of suramin in an in vivo mouse model of hormone-refractory prostate cancer to determine whether a decrease in PSA levels reflects a reduction in tumor growth (volume). The tumors were induced in castrated, athymic nude mice by use of the androgen-independent, tumorigenic human prostate cancer cell line C4-2, which is a subline of the androgen-dependent, parental nontumorigenic cell line LNCaP. We also evaluated the effects of suramin in vitro on cell growth and the expression of PSA messenger RNA (mRNA) in both LNCaP and C4-2 cells. **Methods:** For the in vivo studies, 24 mice were given a subcutaneous injection of 5 x 10⁶ C4-2 cells at each of four sites. Animals (n = 20) with tumor volumes greater than 1 mm³ or less than 5 mm³ were divided equally into two groups. Drug treatment was initiated in one group by administration of 1 mg suramin intraperitoneally, followed by 0.1 mg suramin at 10-day intervals to maintain constant serum levels. Tumor growth and PSA expression levels were monitored. For the in vitro studies, both LNCaP and C4-2 cells were exposed to 100-400 μg/mL suramin, and cell growth was monitored by a quantitative crystal violet assay. PSA mRNA expression was assessed by northern blot analysis in cells treated with either 250 μg/mL suramin, 400 ng/mL dihydrotestosterone (DHT) (positive control), or 0.5-75 μg/mL hydrocortisone (to mimic the clinical use of hydrocortisone during suramin treatment to compensate for the loss of adrenocortical function). In some studies, the combined effect of DHT and suramin on PSA mRNA expression was also evaluated. A two-way analysis of variance was performed to evaluate the treatment differences, and P values were obtained from two-sided tests for statistical significance. **Results:** In vivo, suramin did not significantly affect the growth of androgen-independent C4-2 tumors (relative to the growth of tumors in 5% glucose-treated control animals; P = .76). However, suramin significantly decreased the ratio of PSA level to tumor volume (ng/mL PSA per mm³ of tumor) (P<.001). Mice developed bone metastases in both treatment arms. Suramin affected the in vitro growth of LNCaP cells but not of C4-2 cells. Suramin diminished PSA mRNA expression in both LNCaP and C4-2 cells grown in vitro. Hydrocortisone had no effect on PSA mRNA levels. **Conclusions:** Although suramin inhibited the growth of androgen-dependent LNCaP cells, it did not inhibit the growth of androgen-independent C4-2 cells either in vitro or in vivo. Suramin significantly decreased PSA mRNA expression in both cell lines in vitro and depressed serum PSA levels in mice bearing androgen-independent C4-2 tumors. **Implications:** PSA level should be used with caution as an end point in clinical trials using suramin therapy for hormone-refractory prostate cancer. [J Natl Cancer Inst 1996;88:794-801]
hhibit reverse transcriptase (3), can antagonize the binding of growth factors [platelet-derived growth factor (4), basic fibroblast growth factor (5), transforming growth factor-α, and epidermal growth factor (6)] to their receptors, and can induce cell differentiation (7). Suramin inhibits lysosomal enzymes that degrade glycosaminoglycans (e.g., idurone sulfatase) (8). In addition, it inhibits protein kinase C (9), blocks angiogenesis (10) and cytokine receptor binding (11), interferes with the mitotic process by inhibiting cell motility (6) and urokinase-type plasminogen activator (12), and influences a variety of intracellular and extracellular functions (13).

The last decade has brought increasing attention to prostate cancer. Prostate cancer is recorded as the leading cancer diagnosed and the second cause of cancer death in North American men (14). Metastatic prostate cancer is generally treated by hormone therapy (15). However, once prostate cancer progresses to an androgen-independent state, none of the proposed therapeutic regimens has provided evidence for objective tumor response or a significant survival benefit. Phase I and II clinical trials, based on the rationale of suramin's anti-growth factor activity and its suppression of adrenal androgen synthesis, have led to much interest in and some enthusiasm for suramin as a therapeutic agent in the treatment of hormone-refractory prostate cancer.

Evaluating the response to treatments such as suramin in patients with hormone-refractory prostate cancer constitutes a particular problem. In most patients with hormone-refractory prostate cancer, the bulk of the disease is localized in the bone. Bone scans are not sufficiently sensitive to assess treatment response, partly because repair processes and inflammatory reactions may falsely indicate progressive disease. Patients with measurable tumor alone are not representative of the average patient. Therefore, other criteria of treatment response in patients with nonmeasurable disease are needed.

Difficulties in assessing the treatment benefit, due to the small number of patients with bidimensionally measurable tumor masses, have led to the consideration of trial end points other than time to disease progression; such end points include pain relief and changes in prostate-specific antigen (PSA) expression. PSA, a 34-kd kallikrein-like serine protease, is produced predominantly by prostatic epithelial cells and cleaves high-molecular-weight proteins in the seminal fluid, resulting in liquefaction (16). Serum PSA values reflect total prostate volume, in both normal and malignant tissues (17,18), and are used to monitor and evaluate therapeutic response. The expression of PSA messenger RNA (mRNA) is androgen responsive and can be induced within 6 hours (19).

After radical prostatectomy for localized prostate cancer, PSA levels decrease to normal levels in tumor-free patients, but they remain elevated in patients with residual or occult metastatic disease. Thus, it has been proposed that PSA might be of value in monitoring and predicting treatment response in hormone-refractory disease (20). In an attempt to validate that a reduction in PSA level reflects real diminution of tumor volume and to examine the effects of suramin on PSA expression by prostate cancer cells both in vivo and in vitro, we have used the LNCaP mouse model of human prostate cancer (21,22), which closely mimics the biological, genetic, and molecular events of hormone-refractory prostate cancer progression and dissemination.

In the present study, we assessed the effect of suramin on the growth of the androgen-dependent human prostate cancer cell line LNCaP and its androgen-independent subtype C4-2 both in vivo and in vitro. We also determined whether the expression of PSA and cell growth in vivo were interrelated. We examined the effects of suramin on the growth of and PSA expression by the LNCaP and C4-2 cell lines in vitro (21) under androgen-stimulated and androgen-deprived conditions, as well as under conditions of adrenal hormone substitution (hydrocortisone). Furthermore, we assessed the effects of suramin on the growth of C4-2 tumors in vivo in an androgen-deprived environment (in castrated, athymic nude mice) (22) and correlated the results with serum PSA concentrations in the host. Our in vivo model is ideal for assessing whether suramin may depress PSA expression without affecting prostate cell growth in both androgen-dependent and androgen-independent cancers.

Materials and Methods

Cell Culture and Establishment of Androgen-Independent C4-2 Tumors

LNCaP cells (passages 37-40) and C4-2 cells (passages 20-23) were grown in T-medium (80% Dulbecco's modified Eagle medium obtained from Life Technologies, Inc. [GIBCO BRL], Gaithersburg, MD; 20% F12K obtained from Irvine Scientific Co., Inc., Santa Ana, CA; 3 μg/mL NaHCO3; 100 IU penicillin G; 100 μg/mL streptomycin; 5 μg/mL insulin; 13.6 μg/mL triiodothyronine; 5 μg/mL transferrin; 0.25 μg/mL biotin; and 25 μg/mL adenine) with 5% fetal bovine serum (FBS). All the chemicals were from Sigma Chemical Co., St. Louis, MO, with the exception of FBS, which was obtained from Life Technologies, Inc. The cells were free of Mycoplasma.

Six- to 8-week-old male athymic nude mice (BALB/c strain; Charles River Laboratories, Wilmington, MA) were used as hosts for xenografts of C4-2 tumors for in vivo experiments. They were kept under pathogen-free conditions in laminar flow boxes in accordance with institutional animal usage guidelines and approved protocols. All mice were bilaterally castrated by scrotal incision under methoxyflurane anesthesia. The wound was closed by metal clips (Autoclips; Clay Adams, Parsippany, NJ).

Twenty-four mice were given a subcutaneous injection of 5.0 x 106 C4-2 cells reconstituted in 0.1 mL of T-medium and 10% FBS (27-gauge needle, 1-mL disposable syringe) at four sites per animal. The mice were routinely inspected for physical abnormalities and tumor growth. Those with a tumor volume of greater than 1 mm3 and less than 5 mm3 were eligible for random assignment.

In Vivo Assessment of Suramin Activity

Suramin sodium (hereafter referred to as suramin) was purchased from Miles Inc. (West Haven, CT). It was dissolved in 5% glucose (wt/vol) and was reconstituted to a 10% solution (wt/vol of suramin). A total of 20 mice were found to be eligible for this study (body weight, 18-22 g). Ten castrated animals in the treatment group were initially given an intraperitoneal injection of 1 mg of suramin. Thereafter, they were given an intraperitoneal injection of 0.1 mg of suramin every 10 days on the assumption of a serum half-life of 50 days (7). Using this injection protocol, we determined the steady-state serum suramin concentrations to be 213 μg/mL ± 59 μg/mL (mean ± standard deviation) (performed by Dr. Robert Newman, The University of Texas M. D. Anderson Cancer Center, Houston) (unpublished result). Ten castrated animals in the control group were given an intraperitoneal injection of 5% glucose. Tumor volume was measured every 10 days, before a new treatment cycle was begun.

Measurement of Prostate-Specific Antigen (PSA) Levels

Once tumors became measurable, blood samples for sequential PSA measurements were obtained by dorsal tail vein incision every 10 days. Samples were collected in 75-mm microhematocrit capillary tubes and centrifuged in a microcapillary centrifuge, model MB (IEC, Needham Heights, MA) for 3
minutes at room temperature. The sera were stored at −20 °C until processed further. The microparticle enzyme immunoassay (IMX PSA assay; Abbott Laboratories, Abbott Park, IL) was used for the quantitative measurement of PSA levels in the sera.

In Vitro Assessment of Suramin Activity

For growth assays, LNCaP and C4-2 cells were plated in 24-well plates (Falcon Labware, Becton Dickinson, Franklin Lakes, NJ) at a density of 1 × 10^4 cells per well. Twenty-four hours later, suramin was added at concentrations of 200, 300, and 400 μg/mL. After 2, 4, and 6 days of treatment, cells were fixed with 0.5 mL 1% glutaraldehyde in phosphate-buffered saline for 15 minutes, stained with 0.5% crystal violet, washed with distilled water, and air dried for 24 hours. The dye was eluted in 0.5 mL of Sorensen’s solution (for 1000 mL: 8.967 g trisodium citrate in 305 mL distilled water, 195 mL 0.1 N HCl, and 500 mL 90% ethanol); the solution was shaken for 30 minutes, as described previously (23). The absorption of the eluate at 540 nm was measured with a TriTek Multiscan (Flow Laboratories Inc., Costa Mesa, CA). These in vitro assays were repeated three times.

RNA Blot Analysis

At 24-48 hours after the initial plating of LNCaP and C4-2 cells in T-medium with 5% FBS, the cell cultures at a confluence of 40%-50% were downshifted to either 5% charcoal-stripped FBS or to serum-free conditions of T-medium containing 2% TCM (i.e., totally cellular mouse serum supplement), a serum-free defined medium supplement (Celox Co., Minnetonka, MN). After 24 hours, suramin was added to the cell cultures to a final concentration of 250 μg/mL. Cells were treated with suramin for 3 or 6 days.

We also determined the effects of dihydrotestosterone (DHT) on PSA mRNA expression in LNCaP and C4-2 cells in vitro (positive controls). DHT (400 ng/mL, Sigma Chemical Co.) was added to the cells for 3 and 6 days. In some experiments, suramin (250 μg/mL) was added in combination with DHT for 3 and 6 days to determine if suramin directly antagonizes the action of DHT in stimulating PSA expression by LNCaP and C4-2 cells. Control cell cultures were treated with an equal volume of vehicle (10 μL absolute ethanol and 100 μL 5% glucose).

To determine if hydrocortisone, which is given to patients undergoing suramin treatment to counteract suramin-induced adrenocortical atrophy, is responsible for the suppression of PSA expression, we added 0.5-75 μg/mL hydrocortisone (Sigma Chemical Co.) to test cultures (data not shown). The medium of all cultures in vitro was changed on day 3, and fresh test compounds were added at the time of medium change. These experiments were repeated at least two times.

Total cellular RNA was extracted from cells by the RNAzol B method (Biostat Laboratories Inc., Houston, TX), a single-step purification protocol described by Chomczynski and Sacchi (24). Equal amounts of RNA, as determined by absorbance at 260 nm, were subjected to RNA blot analysis by use of lx TAE buffer (0.04 M Tris base, 0.02 M sodium acetate, and 1 mM EDTA [pH 7.2]). RNAs were cross-linked to the membrane by UV light exposure with a UV-Stratalinker (Stratagene, La Jolla, CA) at 1500 μJ. Hybridizations were performed at 65 °C by use of the Rapid-hyb hybridization buffer (Amersham Life Science Inc., Arlington Heights, IL). 32P-labeled, 1.6-kilobase (kb) PSA complementary DNA (cDNA) and 1.2-kb gliceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA (an RNA loading control), were labeled to a specific activity greater than 1 × 10^8 disintegrations per minute (dpm)/μg. A heat-denatured probe (1 × 10^6 dpm/mL) was used for each hybridization. Membranes were washed consecutively in (a) 2× SSC (0.15 M NaCl and 0.015 M sodium citrate [pH 7.0]) for 30 minutes at room temperature, (b) 2× SSC containing 1% sodium dodecyl sulfate (SDS) twice for 30 minutes at 65 °C, and (c) 0.5× SSC containing 1% SDS once for 30 minutes at 65 °C. Autoradiographs were prepared by exposure of Kodak X-Omat AR film to the membrane at −80 °C with intensifying screens. Autoradiographs of RNA blot analysis for PSA and GAPDH were analyzed, and the levels of PSA mRNA values were normalized to GAPDH by means of the Ambis Optical Imaging System (Ambis Inc., San Diego, CA).

Statistical Analysis

For the comparison of tumor growth and PSA expression per tumor volume between the two treatment groups, a two-way analysis of variance (ANOVA) was performed using SPSS for Windows (SPSS Inc., Chicago, IL). Prior to analysis, the data were log-transformed to stabilize the variance of geometrically expanding variables (tumor volume and PSA level). Repeated-measures analysis (e.g., nesting animals within treatments) was considered; however, the decreasing number of animals at each treatment cycle removed this option. We calculated an unbalanced nested design with the same results as the two-way analysis. Box plots were calculated by SPSS with median center, box at the 25th and 75th percentiles, and lines at 1.5 times the interquartile range. The numbers in Figs. 1 and 2 denote the outlying observations as generated by SPSS and are labeled with (+) and (−). The significance of the effects of suramin on cell growth curves in vitro was also determined by two-way ANOVA (concentration and drug) with no transformation of the data. In addition, Student’s t tests were performed on data at selected days. The cell growth data are expressed as mean values ± standard deviation with P values obtained from the two-way analysis. All P values were generated from two-sided tests for statistical significance.

Results

In Vivo Assessment of Suramin Activity

Injection of 5.0 × 10^6 C4-2 cells into androgen-deprived (castrated) mice resulted in tumors in 22 (91.67%) of 24 animals. Two of these mice were not eligible for random assignment to a treatment group because they had an initial tumor volume of greater than 5 mm^3. The remaining 20 animals were assigned randomly to either the control group (n = 10) or the suramin treatment group (n = 10). The mean number of tumors per animal at the time of treatment assignment was 1.5 in the control group and 1.64 in the suramin-treated group.

The mean-tumor-growth curve of the group treated with suramin did not differ significantly (P = .76) from that of the control group treated with intraperitoneal injections of 5% glucose only (Fig. 1). PSA measurements, however, indicated lower PSA levels in suramin-treated animals. An analysis of the ratio of the PSA level to tumor volume demonstrated a highly significant difference (P<.001) between the control and suramin-treated groups, showing that less PSA is synthesized and/or secreted per volume of the tumor after suramin treatment (Fig. 2). Survival of the animals did not differ between the two treatment groups beginning at the time they started the treatment (data not shown). Two animals in the treatment group (20%) and three animals in the control group (30%) developed paraplegia due to spinal compression by osteoblastic metastases.

In Vitro Assessment of Suramin Activity

Suramin (400 μg/mL) significantly (P<.001) suppressed the growth of the androgen-dependent and nontumorigenic LNCaP cell line (Fig. 3, A). Lower doses of suramin (200 and 300 μg/mL) appeared to decrease transiently the growth of LNCaP cells in vitro day 4 but not at day 6. Suramin, however, was ineffective (P = .509) in suppressing the growth of the androgen-independent, tumorigenic, and metastatic C4-2 cell line in vitro (Fig. 3, B).

RNA blot analysis of LNCaP and C4-2 cells was performed to assess the levels of PSA mRNA expression in cells treated with suramin (Fig. 4). Control levels of PSA mRNA expression were the same at day 3 and day 6; therefore, only levels at day 3 are shown. Under serum-free culture conditions, the steady-state levels of PSA mRNA expression were decreased by suramin at days 3 and 6 in both the androgen-dependent parental LNCaP cells and the androgen-independent C4-2 cells (Fig. 4, A). As
Fig. 1. Effects of suramin on growth of androgen-independent human prostate tumors in vivo. Box plot of a two-way analysis of variance of tumor volume in the suramin treatment group compared with the control group. Athymic nude mice were initially given an intraperitoneal injection of 1.0 mg suramin or an equal volume of 5% glucose and then given every 10 days an intraperitoneal injection of 0.1 mg suramin or an equal volume of glucose. Note that there was no difference between the two treatment groups ($P = .76$). Median center, box at 25th and 75th percentiles, lines at 1.5 times the interquartile range. Numbers denote the outlying observations as generated by SPSS and are labeled with (*) and (+).

Fig. 2. Effects of suramin on ratio of prostate-specific antigen (PSA) expression to tumor volume by androgen-independent human prostate cancer tumors in vivo. Box plot of a two-way analysis of variance of mean PSA/tumor volume in relation to injections in 10-day intervals. Note significant ($P<.001$) difference between the curve for the animals treated with suramin and that for animals in the control group. Median center, box at 25th and 75th percentiles, lines at 1.5 times the interquartile range. Number denotes the outlying observation as generated by SPSS and is labeled with (+).
Radiographic evidence of regression of bone lesions was seen reported to have had partial resolution of their disease and no consistent with the findings obtained from phase II clinical trials (25, 26).

Although the tumor volume in the mice treated with Suramin did not decrease significantly, PSA levels decreased significantly in these mice.

Animals treated with suramin invariably exhibited a decrease in serum PSA levels. These levels rebounded somewhat after several weeks (weeks 9 and 10), pointing to a possible progression of the disease. The levels of serum PSA when the disease progressed, however, were not uniformly as high as those before treatment. These findings are consistent with the clinical observation that some patients treated with suramin do not show the expected logarithmic increase in serum PSA levels when their disease progresses. The decrease and subsequent rebound of serum PSA expression in vivo may be due to one or more of the following mechanisms: 1) reduction of PSA mRNA and protein expression by suramin, 2) selection of a less differentiated tumor cell population with quantitatively less PSA production per tumor burden (27), 3) selection of suramin-resistant prostate cancer cell clones or decreased suramin uptake (28), or 4) activation of an alternative PSA-producing pathway such as the androgen receptor-independent PSA-stimulating autocrine factor (PSAF) pathway (29).

Suramin inhibited LNCaP cell proliferation in vitro, as described by La Rocca et al. (30) and Berns et al. (31), but it did not affect the growth of hormone-refractory C4-2 prostate cancer cells in culture as seen in this study. This finding stands in contrast to observations of a dose-dependent growth inhibition in androgen-independent PC-3 (30, 32) and DU-145 (6, 30, 33) cells. These results imply that, among androgen-independent human prostate cancer cells, a heterogeneity may exist with respect to suramin inhibition.

### Discussion

In this study, we examined the effects of suramin on the androgen-independent, metastatic, and PSA-expressing C4-2 cells and tumors and the androgen-dependent, nonmetastatic, and PSA-expressing LNCaP cells in a mouse model (21, 22) of human prostate cancer. Suramin did not affect tumor volume or survival, and it did not prevent the development of osseous metastases in this in vivo model. These experimental results are consistent with the findings obtained from phase II clinical trials in which only a few patients with measurable tumors were reported to have had partial resolution of their disease and no radiographic evidence of regression of bone lesions was seen (25, 26). Although the tumor volume in the mice treated with suramin did not decrease significantly, PSA levels decreased significantly in these mice.
RNA blot analysis of androgen-dependent LNCaP and androgen-independent C4-2 prostate cancer cells treated with suramin in vitro indicated a reduction of PSA mRNA expression in both. PSA mRNA expression is regulated by different factors, such as DHT, extracellular matrix (34), and PSAF (29). Suramin has been reported to lower adrenal androgens in humans (30) and circulating testosterone levels in rats (35). When castrated rats received androgen supplementation, however, there was no
difference in prostate weights between suramin-treated rats and a control group (35). Hydrocortisone, which in clinical trials is administered to counteract suramin-induced adrenocortical atrophy, did not interfere with the steady-state levels of PSA mRNA expression (data not shown). Thus, if this LNCaP mouse model is applicable to human prostate cancer conditions, we can conclude that the decreased PSA expression observed in patients treated with suramin may be the result of a direct effect of suramin on PSA mRNA expression rather than the result of suramin inhibition of tumor growth.

There is a growing body of experimental evidence that PSA gene expression has underlying complex control mechanisms and that steady-state levels of PSA mRNA are increased by androgens (18) and decreased by epidermal growth factor and by the activation of protein kinase C (36,37). Protein kinase C is a family of protein serine–threonine kinases that mediate intracellular signal transduction. Recently, suramin has been reported to interfere with the protein kinase C pathway (9,38), thus providing a possible explanation for the observed suramin-induced decrease in PSA mRNA expression. Although it appears that in LNCaP cells the suramin-induced decrease in PSA expression correlates with growth inhibition, the possible significance of this inhibition in vivo remains unclear, particularly in view of the fact that suramin concentrations reached in vivo (213 μg/mL ± 59 μg/mL, mean ± standard deviation) were below the threshold level inhibiting LNCaP cell growth in vitro (Fig. 3, A). When C4-2 cells were treated with suramin, their growth was not affected, yet PSA mRNA expression remained low in these cells. Furthermore, as La Rocca et al. (30) observed in LNCaP cells, we also found that PSA protein synthesis and/or secretion were also markedly reduced in the culture medium of LNCaP cells. However, we have extended their observation by showing that an androgen-independent, PSA-secreting LNCaP subline, C4-2, when treated with suramin also shows a decreased PSA secretion (data not shown).

The clinical implications of our findings are as follows: 1) Suramin inhibition of PSA expression may not be mediated by serum factors alone because we have observed a suramin-induced decrease in PSA levels under both serum-containing and serum-free conditions. 2) Suramin may exert a direct action (rather than mediated by testicular androgen or adrenal steroids) in decreasing the PSA expression by prostate cancer cells. 3) Our results cannot completely exclude the possibility that, under some clinical conditions, suramin may depress both growth and PSA expression by prostate cancer cells. This possibility is reflected by the observation that suramin depressed the growth of two human androgen-independent prostate cancer cell lines, PC-3 (30,32) and DU-145 (6,30,33). 4) PSA-secreting, androgen-independent prostate cancer may not be affected uniformly by suramin treatment, as measured by tumor growth and PSA secretion.

In summary, PSA is a valuable serum marker for prostate cancer, and pretreatment PSA values correlate with pathologic stage and grade (39,40), tumor volume (17), and presence or absence of metastases. PSA is the single most significant predictor of disease outcome after radiation therapy for local–regional prostate cancer (41). Our data from the experimental LNCaP mouse model of human prostate cancer, which for the first time permits the study of PSA expression by hormone-refractory prostate cancer in an androgen-deprived host microenvironment, suggest that suramin does not inhibit the proliferation of hormone-refractory C4-2 tumors, but it does decrease PSA mRNA expression in vitro as well as in the in vivo PSA production by tumors. Thus, PSA should be used with caution as an end point in clinical trials of suramin for hormone-refractory prostate cancer and in the evaluation of suramin treatment response.

References

The Familial Kidney Cancer Program of the National Cancer Institute is interested in seeing:

- Members of families with von Hippel-Lindau disease
- Members of families with two or more members affected with papillary renal carcinoma
- Members of families with three or more members affected with clear cell renal carcinoma

The Familial Kidney Cancer Program of the NCI will provide genetic and clinical diagnosis and treatment for selected families.

For more information please contact:

Berton Zbar, M.D.,
301-846-1288 (phone)
301-846-6145 (fax)
zbbar@ncifcrf.gov (e-mail)

or

W. Marston Linehan, M.D.
301-496-6353 (phone)
301-402-0922 (fax)
wml@helix.nih.gov (e-mail)