[CANCER RESEARCH 50, 4190-4194, July 15, 1990]

Antiproliferative and Antitumor Effects of α-Interferon in Renal Cell Carcinomas: Correlation with the Expression of a Kidney-associated Differentiation Glycoprotein[1]

David M. Nanus, Lawrence M. Pfeffer, Neil H. Bander, Sanjeev Bahri, and Anthony P. Albino[2]

Laboratory of Mammalian Cell Transformation [D. M. N., S. B., A. P. A.], Division of Solid Tumor Oncology, Department of Medicine [D. M. N.], and Laboratory of Human Cancer Immunology [N. H. B.], Memorial Sloan-Kettering Cancer Center, New York, New York 10021; The Rockefeller University, New York, New York [L. M. P.]; and Division of Urology, Department of Surgery, New York Hospital-Cornell University Medical Center, New York, New York [N. H. B.]

ABSTRACT

Human leukocyte α-interferon (IFN-α) has significant antitumor activity in advanced renal cell carcinoma (RC), with approximately 15% (range, 5 to 29%) of patients subjected to IFN-α therapy exhibiting a major objective response. We assayed 16 RC cell lines for intrinsic sensitivity to the growth-inhibitory effects of recombinant IFN-α. Similar to results observed in patients, cultured RCs could be divided into those that are inhibited by IFN-α and those that are not. In addition, the IFN-α-sensitive or -resistant phenotype of cultured RCs was correlated with surface expression of six unrelated kidney-associated differentiation antigens. The expression of one antigen, a M_r 160,000 glycoprotein (gp160), was found to correlate with resistance to IFN-α. Proliferation of seven RC cell lines expressing gp160 ($gp160^+$) was not significantly inhibited by IFN-α at concentrations as high as 3000 units/ml. In contrast, proliferation of eight of nine RC cell lines lacking expression of gp160 ($gp160^-$) was markedly inhibited by IFN-α. The effect of IFN-α on $gp160^+$ and $gp160^-$ RC xenografts in *nu/nu* mice was examined. In separate experiments, two $gp160^+$ RC cell lines and five $gp160^-$ RC cell lines were injected s.c. into *nu/nu* mice; one half of the mice were subsequently treated with 10^6 units of IFN-α i.p. 3 times a wk, and one half received no IFN-α. Tumors appeared at the sites of inoculation in all mice given injections of $gp160^+$ RC cell lines within 10 to 25 days regardless of INF-α therapy. Mice given injections of $gp160^-$ RC cell lines, but not receiving IFN-α, also formed tumors. In contrast, $gp160^-$ RC cell lines injected into mice that were treated with IFN-α exhibited a marked sensitivity, as demonstrated by either no tumor formation or delayed tumor formation. We conclude that the absence of gp160 expression by RCs may be predictive of sensitivity to the antitumor effects of IFN-α and, thus, provide a basis for identifying IFN-responsive patients.

INTRODUCTION

RC[3] is the most common neoplasm of the adult kidney, with over 20,000 new cases each year in the United States (1, 2). Approximately 30 to 40% of patients with RC present with locally advanced or metastatic disease (3), while another 30% relapse following nephrectomy for early stage disease (4). Chemotherapeutic agents are ineffective in RC patients. In 1983 and 1985, Quesada *et al.* (5, 6) reported the clinical efficacy of the biological response modifier human leukocyte IFN-α in metastatic RC. Subsequently, numerous clinical trials with purified and recombinant IFN-α reported major responses in from 5 to 29% of patients treated, with a median duration of response ranging from 3 to 16 mo (7, 8). The cumulative clinical experience with IFN-α indicates that a subset of RC patients are sensitive to this therapy. However, it has been impossible to predict which patients will respond to IFN-α. Our previous studies have demonstrated that cultured and noncultured RCs can be subclassified by virtue of differing expression of a series of kidney-associated differentiation antigens to which we have generated mAbs (9-13). As a consequence, we have speculated that differential expression of one or more of these kidney antigens may be useful in identifying RCs with different biological and clinical characteristics (12, 14), for example, IFN-α sensitivity. In this paper, we have defined the relationship between IFN-α sensitivity and specific antigen expression using tissue culture and mouse model systems. The results showed that (*a*) subsets of cultured RCs are either sensitive or resistant to the antiproliferative effects of IFN-α; (*b*) expression of a gp160 correlated with resistance to IFN-α, and, conversely, that lack of expression of gp160 correlated with sensitivity to IFN-α; and (*c*) the correlation of IFN-α-sensitive or -resistant phenotypes with gp160 expression is also observable *in vivo* using a mouse model.

MATERIALS AND METHODS

Cell Lines and Tissue Culture. Short-term cultures of normal proximal tubule cells (PT1, PT2) and renal cancer cell lines were derived as previously described (15). RC cell lines were from either primary (*i.e.*, SK-RC-1, 4, 35, 44, 49) or metastatic (*i.e.*, SK-RC-2, 12, 17, 26, 28, 29, 38, 39, 41, 42, 45; adrenal, 3; lung, 3; bone, 2; soft tissue, 2; brain, 1) renal cancers. Cultures were maintained in MEM supplemented with 2 mM glutamine, 1% nonessential amino acids, 100 units/ml of streptomycin, 100 units/ml of penicillin, and 7.5% FBS.

Serological Reagents. The mouse mAbs used are summarized in Table 1.

Serological Assays. The Protein A and anti-mouse immunoglobulin hemadsorption assays were performed as described (16). Indicator cells were prepared by conjugating the immunoglobulin fraction of rabbit anti-mouse heavy chain (Dako Corp., Santa Barbara, CA) to human 0^+ erythrocytes with 0.01% chromium chloride. Assays were performed in Falcon 3040 microtest plates (Falcon Labware, Oxnard, CA). Target cells (plated 1 to 2 days previously) and serial antibody dilution were incubated for 1 h at room temperature and then washed, and human erythrocyte indicator cells were added for 45 min. Target cells were washed again to remove nonadherent indicator cells. Titers were defined as the antibody dilution showing 20% positive (rosetted) target cells as evaluated by light microscopy.

Immunoprecipitation Analysis. Cells were radiolabeled by metabolic incorporation of [^{35}S]methionine (1000 Ci/mmol; New England Nuclear, Boston, MA) using 250 μCi in 10 ml of methionine-free MEM containing 7.5% FBS for 16 h. Labeled cells were extracted as described (17). Immunoprecipitation was carried out by mixing a portion of the cell extract (10×10^6 cpm) with 1 μl of undiluted ascites fluid of mAb F33 (anti-gp160). Immune complexes were isolated with Protein A-

Received 12/21/89; revised 3/9/90.
The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
[1] This work was supported in part by the Charles H. Revson Foundation, the American Philosophical Society, a Young Investigator Award from the American Society of Clinical Oncology, The Society of Memorial Sloan-Kettering Cancer Center, and NIH Research Grant GM36716. D. M. N. is a Charles H. Revson Clinical Scholar in Biomedical Research, an American Philosophical Society Daland Fellow, and a 1988 Young Investigator of the American Society of Clinical Oncology. L. M. P. in a Scholar of the Leukemia Society of America. S. B. is a recipient of an American Urological Association Summer Student Fellowship.
[2] To whom requests for reprints should be addressed, at Memorial Sloan Kettering Cancer Center, Box 167, 1275 York Avenue, New York, NY 10021.
[3] The abbreviations used are: RC, renal cell carcinoma; IFN-α, α-interferon; PT, proximal tubule; gp160, M_r 160,000 glycoprotein p170, M_r 170,000 protein; mAbs, monoclonal antibodies; MEM, Eagle's minimal essential medium; FBS, fetal bovine serum.

Downloaded from http://aacrjournals.org/cancerres/article-pdf/50/14/4190/2439840/cr0500144190.pdf by guest on 28 May 2024

Table 1 *Monoclonal antibodies*

mAb designation (immunoglobulin subclass)	Antigen (M_r)	Site of expression	Ref.
F33 (γ1)	160,000	Glom,[a] PT	
S22 (γ1)	115,000	RC only	10
S23 (γ1)	120,000	PT, LH	10
T43 (γ1)	85,000	PTc	26
F23 (γ2a)	140,000	PT	10
F31 (IgM)	Glycolipid	PTs	13

[a] Glom, glomerulus; LH, loop of Henle; PTc, pars convoluta of the proximal tubule; PTs, pars recta of the proximal tubule.

Sepharose-C14B (Pharmacia, Inc., Piscataway, NJ), and the labeled components were detected by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and fluorography as described (17).

Interferon Effect on Proliferation. Cell lines were plated at 1 × 10^5 cells/25-cm^2 flask in Eagle's MEM with FBS. After 1 day the cells were refed with MEM with FBS containing recombinant IFN-α (r-metHuIFN-Con_1), a consensus analogue of the most frequent amino acid residues known to occur in subspecies of α-interferons (Amgen, Inc., Thousand Oaks, CA) (18) at 100, 300, 1000, and 3000 units/ml. Control cultures were refed with MEM with FBS and without IFN-α. On Day 7, cells were washed once with phosphate-buffered saline and harvested by trypsinization for 5 min at 37°C. Cell counts were performed on a Coulter Counter (Coulter Electronics, Hialeah, FL). The ratios of the cell number on Day 7 to that on Day 0 in IFN-α-treated cultures were expressed as a percentage of the same ratio (Day 7/Day 0) in untreated control cultures.

Renal Cancer Xenografts. The renal cancer cell lines used above were assayed for their ability to form tumors following inoculation of 1 × 10^6 cells into the flank of 4- to 6-wk-old female Swiss-*nu/nu* mice. Cell lines that were tumorigenic were then used to determine the effect of IFN-α on renal cancer xenografts *in vivo*. For each cell line tested, 8 to 10 mice were inoculated s.c. in the right flank with a single cell suspension of 1 × 10^6 cells in 0.2 ml of MEM without FBS. Subsequently, one half of the mice in each group received 1 million units of recombinant IFN-α (r-metHuIFN-Con_1) in 0.5 ml of MEM without FBS i.p. IFN-α therapy was initiated at the time of injection of RC cells and continued on a thrice weekly schedule. One half the mice in each experiment (the control group) received no IFN-α therapy. All mice were examined for tumor formation 3 times a wk. Individual mice in each trial with a different RC cell line were sacrificed when (*a*) the tumors reached a diameter of 1 cm; (*b*) the tumors became ulcerated; (*c*) no tumor had formed after 150 days; or (*d*) the mice developed an opportunistic infection (bacterial or fungal) unrelated to the treatment. Serum levels of IFN-α were not monitored.

RESULTS

Growth Inhibition Assays. The effects of IFN-α on the proliferation of two normal PT cell cultures (PT1, PT2) and 16 RC cell lines were analyzed at increasing concentrations ranging from 100 to 3000 units of IFN-α/ml. Both normal PT cultures and 8 RC cell lines were found to be relatively resistant to the antiproliferative effect of IFN-α. These cell lines showed <20% inhibition of growth with IFN-α concentrations as high as 3000 units/ml (Fig. 1*A*). In contrast, 7 other RC cell lines showed >50% growth inhibition with IFN-α concentrations of ≤1000 units/ml when compared to untreated cultures (Fig. 1*B*). A 50% inhibition of cell proliferation could also be demonstrated in an eighth RC cell line (SK-RC-29), but required an IFN-α concentration of 3000 units/ml. Cytotoxicity at high concentrations of IFN-α (≥1,000 units/ml) was noted in several highly sensitive cell lines (*i.e.*, SK-RC-2, 41, 42, 44, 49).

gp160 Expression and IFN-α Sensitivity. The antigenic phenotype of each cell line used above was determined by immunorosetting assays and a panel of mAbs generated to 6 distinct and unrelated kidney-associated differentiation antigens (see Table 1). This panel of antigens, consisting of 5 surface glycoproteins and one surface glycolipid, represents a range of kidney-specific markers that are expressed universally in normal PT cells (except for S22 which is only expressed in RCs), and variably in cultured and noncultured RCs. As shown in Table 2, the expression of this set of antigens was correlated with the biological phenotype of resistance or sensitivity to the antiproliferative action of IFN-α. There was no demonstrable correlation with 5 of the 6 antigens. However, one antigen, a surface gp160 defined by mAb F33, did correlate with IFN-α effect. The PT1, PT2, and 7 RC cell lines that were resistant to IFN-α expressed high levels of gp160 (Fig. 1*A*; Table 2). In contrast, 8 of 9 other RC cell lines that did not show detectable expression of gp160 were markedly sensitive to the antiproliferative effects of IFN-α (Fig. 1*B*; Table 2). These data suggested that the lack of gp160 expression by RC cell lines is predictive for sensitivity to the antiproliferative effect of recombinant IFN-α, and, conversely, that resistance correlates with the expression of gp160.

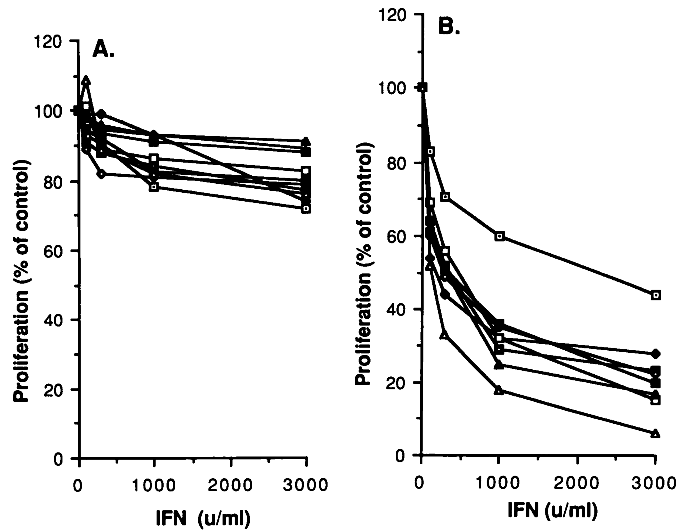

Fig. 1. α-Interferon effects on renal carcinoma cell proliferation. Sixteen renal cancer cell lines and two short-term cultures of normal proximal tubule cells were plated at 1 × 10^5 cells/25-cm^2 flask (Day 0) and refed with medium containing recombinant IFN-α at 100, 300, 1000, and 3000 units/ml (Day 1). Control cultures were refed with medium without IFN-α. On Day 7, the cells were harvested by trypsinization and counted. The ratios of the cell number on Day 7 to that on Day 0 in IFN-treated cultures were expressed as a percentage of the ratio in untreated control cultures. Values represent an average of at least two separate experiments on each cell line. The designation SK-RC denotes renal cancer cell lines established at Sloan-Kettering Institute; PT denotes short-term cultures of normal proximal tubule cells. *A* (resistant cells): SK-RC-26 (□); SK-RC-28 (◆); SK-RC-35, (■); SK-RC-45 (■); SK-RC-4 (■); SK-RC-12 (□); SK-RC-1 (▲); SK-RC-38 (▲); PT-1 (■); PT-2 (+). *B* (sensitive cells): SK-RC-29 (□); SK-RC-2 (◆); SK-RC-17 (■); SK-RC-39 (◇); SK-RC-42 (■); SK-RC-41 (□); SK-RC-49 (▲); SK-RC-44 (▲).

gp160 Expression by Immunoprecipitation. In order to confirm the presence or absence of gp160, RC cell lines were radiolabeled with [^{35}S]methionine, and cell lysates were subjected to immunoprecipitation with mAb F33. Fig. 2 shows representative immunoprecipitation results. In confirmation of the immunorosetting analysis, a M_r 160,000 protein was present in gp160$^+$ RC cell lines and not detectable in gp160$^-$ RC cell lines.

Renal Cancer Xenografts. To assess the potential applicability of this finding to patient therapy, we used an *in vivo* mouse model to determine (*a*) whether *in vitro* sensitivity to the antiproliferative action of IFN-α correlated with antitumor effects of IFN-α *in vivo*, and (*b*) whether the correlation of resistance

Downloaded from http://aacrjournals.org/cancerres/article-pdf/50/14/4190/2439840/cr0500144190.pdf by guest on 28 May 2024

Table 2 *Expression of kidney differentiation antigens and α-interferon effect*

Expression of surface glycoproteins was determined by erythrocyte rosetting assays: ▣, a reactive mAb titer of ≤1/10,000; ■, a reactive mAb titer of ≥1/100,000; and ○, no reactivity.

Cell lines	Antibodies S22	S23	T43	F23	F31	F33	IFN-α[a]
PT1	▣	■	■	■	▣	■	R
PT2	▣	■	■	■	▣	■	R
SK-RC-1	■	■	■	■	▣	■	R
SK-RC-4	■	■	▣	▣	○	■	R
SK-RC-12	ND[b]	■	■	○	○	■	R
SK-RC-26	○	■	■	▣	▣	■	R
SK-RC-28	○	■	■	○	▣	■	R
SK-RC-38	ND	■	■	■	■	■	R
SK-RC-45	■	■	■	■	▣	■	R
SK-RC-35	ND	■	■	○	▣	○	R
SK-RC-2	■	○	■	■	▣	○	S
SK-RC-17	ND	■	■	■	■	○	S
SK-RC-29	○	■	■	■	▣	○	S
SK-RC-39	ND	■	■	■	▣	○	S
SK-RC-41	ND	■	■	▣	▣	○	S
SK-RC-42	○	■	■	■	▣	○	S
SK-RC-44	ND	■	■	▣	■	○	S
SK-RC-49	ND	■	■	■	■	○	S

[a] IFN-α effect determined by growth inhibition assays (see "Materials and Methods"): S, >50% inhibition of cell growth as compared with untreated control cultures at an IFN-α concentration of <1000 units/ml (except for SK-RC-29, where 50% inhibition occurred at 3000 units/ml); R, <20% inhibition of cell growth as compared with untreated control cultures at an IFN-α concentration up to 3000 units/ml.

[b] ND, not determined.

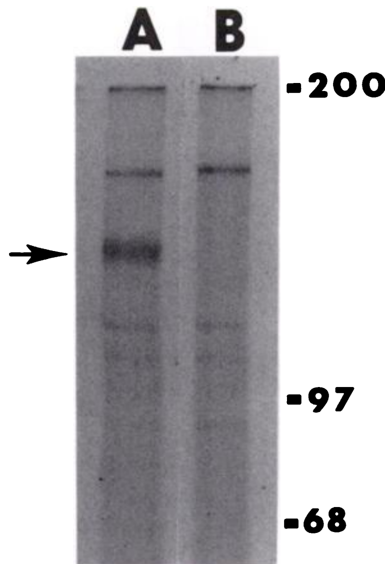

Fig. 2. Immunoprecipitation of gp160. Autoradiograms of immunoprecipitates obtained with mAb F33 (anti-gp160) and extracts of [^{35}S]methionine-labeled SK-RC-45 (gp160$^+$) and SK-RC-44 (gp160$^-$) as analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. *Lane A*, SK-RC-45; *Lane B*, SK-RC-44. *Arrow* shows gp160.

to IFN-α and expression of gp160 seen *in vitro* was valid *in vivo* in a mouse model. The experiment was designed to simulate the clinical application of IFN-α in the treatment of RCs, in which patients receive IFN-α systemically and not directly to the tumor mass. Therefore, unlike the *in vitro* experiments discussed above in which IFN-α was added directly to the RC cells, here we determined the effects of systemically administered IFN-α on the growth of subcutaneous human renal cancer xenografts. Since not all RC cell lines are tumorigenic in *nu/nu* mice, we first assessed the ability of gp160$^+$ and gp160$^-$ RC cell lines to form subcutaneous tumors in athymic mice (see "Materials and Methods"). Upon injection of 1×10^6 RC cells, 8 of 16 RC cell lines were consistently tumorigenic, forming a 0.2- to 0.5-cm^3 tumor within 6 wk; the remaining 8 showed negligible growth under similar conditions. Of these 8 tumorigenic RC cell lines, 2 were gp160$^+$ and 6 were gp160$^-$, and 7 of these were used for mouse experiments (see Table 3). Tumors appeared at the sites of inoculation in all mice receiving injections of gp160$^+$ RC cells within 10 to 15 days (median values: range, 10–25 days), regardless of whether they were treated or untreated with IFN-α. Mice given injections of gp160$^-$ RC cells, but not receiving IFN-α, also formed tumors. In contrast, IFN-α exhibited a marked antitumor effect in the mice injected with gp160$^-$ RC cells; the mice in this group manifested either no tumor formation or substantially delayed tumor formation. These data indicate that, similar to previous experience in clinical trials with RC patients, a subset of RCs is sensitive to IFN-α therapy. More importantly, these results show that each of the gp160$^-$ RC are sensitive to IFN-α in an *in vivo* setting.

DISCUSSION

IFN-α is an effective form of therapy in the treatment of a subset of metastatic RCs with the proportion of patients responding in clinical trials of IFN-α in RC averaging approximately 15% (range, 5 to 29%) (7, 8). However, the majority of RCs are unresponsive to the antitumor effects of IFN-α. Whether the biological basis for this differential sensitivity to IFN-α is a function of the individual RC or of the host is obscure. The mode of action of IFN-α is presumed to involve both direct cytotoxic and antiproliferative effects on tumor cells and indirect effects that facilitate immune detection by the host (*e.g.*, increased natural killer cell and monocyte activity, induction of tumor cell surface antigens, etc.) (19). Clearly, an ability to discriminate and define cellular as well as host mechanisms involved in the antiproliferative effects of IFN would be valuable. Moreover, identification of the subset of RCs that would be inhibited by IFN-α would provide not only a model system with which to study the mechanism of the antitumor action of IFN-α, but potentially a diagnostic procedure allowing a more precise tailoring of treatment for individual patients. In this paper, we showed that (*a*) IFN-α has direct antiproliferative and antitumorigenic effects on cultured RCs; (*b*) RCs can be divided into those that are sensitive to the effects of IFN and

Table 3 *Antiproliferative effect of α-interferon on renal cancer xenografts*

Cell line	gp160 expression[a]	Median no. of days to tumor formation[b] −IFN-α	+IFN-α[c]
SK-RC-01	+	14	15
SK-RC-45	+	10	10
SK-RC-17	−	6	>77[d]
SK-RC-39	−	30	>73[e]
SK-RC-42	−	40	NTF[f,g]
SK-RC-44	−	7	NTF[h]
SK-RC-49	−	5	NTF[i]

[a] gp160 expression determined by immunorosetting assays: +, detectable expression at mAb titers of >1/100,000; −, no detectable expression (see Table 2).

[b] Tumor formation assessed as the initial presentation of a subcutaneous nodule ≥2 mm which continued to enlarge.

[c] IFN-α, treatment with 1 million units of recombinant IFN-α (r-metHuIFN-Con_1) i.p. on a Monday-Wednesday-Friday schedule.

[d] In this group of 4 mice, tumors were detected at Days 19 and 59, and 2 mice were sacrificed at Day 98 with no evidence of tumor formation.

[e] In this group of 3 mice, tumors were detected at Days 66 and 73, and one mouse was sacrificed at Day 82 with no evidence of tumor formation.

[f] NTF, no evidence of tumor formation.

[g] This entire group of 4 mice was sacrificed at Day 150 with NTF.

[h] This group of 5 mice was sacrificed at Days 33, 94, 122, 122, and 122 with NTF.

[i] This group of 4 mice was sacrificed at Days 49, 86, 86, and 86 with NTF.

Downloaded from http://aacrjournals.org/cancerres/article-pdf/50/14/4190/2439840/cr0500144190.pdf by guest on 28 May 2024

those that are resistant; and (*c*) the phenotype of resistance or sensitivity can be correlated with the expression of one cell surface M_r 160,000 glycoprotein.

IFN-α markedly inhibited the growth of a subset of RC cell lines growing as monolayers *in vitro*. While IFN-α has direct antiproliferative effects on these RC cell lines, the specific types of biochemical derangements have not yet been defined. The concentrations of IFN-α that inhibited the growth of these RC cell lines *in vitro* were within a range that is equivalent to mean serum concentrations achieved in patients with RC after a standard IFN-α protocol (*i.e.*, 100 to 1000 units/ml) (20). When the same set of RC cell lines was grown, not in tissue cultures, but as tumor xenografts in mice, their proliferation was also markedly inhibited by systemically administered (*i.e.*, i.p.) IFN-α. Therefore, regardless of whether IFN-α was added directly to tumor cells *in vitro* or indirectly to the tumor *in vivo*, the biological results, *i.e.*, the inhibition of proliferation, were identical. While it is more difficult to define the precise effects of IFN-α given systemically to mice carrying a RC xenograft, it would be reasonable to assume that IFN-α had a direct antiproliferative effect which was sufficient to inhibit tumor formation by RC cells.

Immunological analysis indicated that IFN-α-sensitive and -resistant RCs expressed a similar panel of kidney-associated antigens and, thus, have a common antigenic phenotype. However, there was one notable exception. RCs that were resistant to IFN-α expressed gp160 on their cell surfaces, while those RCs that were sensitive to IFN-α did not express gp160 (Table 2). The gp160 molecule, which we have previously characterized (9, 10, 12), is normally expressed on the cell surfaces of the glomerulus and PT cell of the human nephron and on the cell surface of ≈80% of cultured and noncultured RCs (Footnote 4; Ref. 21). A wide range of normal and neoplastic tissues of nonkidney origin do not express detectable amounts of gp160 (10). However, gp160 can be detected on a subset of sarcomas (10). Immunoprecipitation studies using radiolabeled cell lysates confirmed that gp160$^-$ RC cell lines did not have detectable amounts of this protein. Consequently, based on differential expression of a surface glycoprotein, a prediction could be made as to which RC cell lines would respond to the antiproliferative effects of IFN-α. This predictive correlation was shown to be valid both *in vitro* in cell culture and *in vivo* in a mouse model system.

The biochemical function of gp160 is not yet known. Therefore, whether gp160 is directly or indirectly involved in the resistance of RC to IFN-α, or merely cophenotypes fortuitously with another gene(s) product which does convey resistance to IFN-α remains to be determined. We considered the possibility that gp160 is related to the multiple drug resistance gene product. This consideration was based on the facts that (*a*) p170 is similar in molecular weight to gp160 (22), (*b*) p170 is also expressed at high levels in renal tissues (23), and (*c*) expression of p170 correlates with resistance of RCs to the cytotoxic effects of certain chemotherapeutic agents, *e.g.*, Adriamycin (24). Immunological analysis, however, showed a different pattern of expression of gp160 and p170 in RC cell lines and renal tissues: gp160 and p170 were not coordinately expressed *in vitro* in the RCs used in this study (data not shown). Moreover, *in vivo*, glomerular epithelium was gp160$^+$ but p170$^-$ (9, 25). Thus, it is unlikely that gp160 and the multiple drug resistance p170 are identical proteins.

In conclusion, these results indicate that the absence of surface gp160 protein expression on RC cell lines correlates *in vitro* with a phenotype of sensitivity to the antiproliferative effects of IFN-α. If a similar correlation exists *in vivo*, then the absence of gp160 expression in a biopsied renal tumor may identify those patients with a high probability of achieving a positive clinical response to IFN-α therapy. Consequently, a prospective clinical trial is presently in progress at Memorial Sloan-Kettering Cancer Center and New York Hospital to test this hypothesis. Retrospective studies attempting to correlate gp160 phenotype with patients previously treated with IFN-α were not possible as gp160 immunoreactivity is not detectable in tissues that have been formalin fixed and paraffin embedded. If our hypothesis is validated, patients unlikely to derive therapeutic benefit could be spared the toxicity of IFN-α treatment and could be offered alternative therapy without delay. Moreover, patients at high risk of failure (*e.g.*, those with resected Stage III disease) who have gp160$^-$ tumors could be offered adjuvant therapy early, at a time when they are theoretically most likely to benefit.

[4] N. H. Bander, unpublished data.

ACKNOWLEDGMENTS

We thank Maude Blundell, Maria Davis, Harold Mcquilla, and Thomas Hunter for expert technical assistance; Dr. J. O'Brien for assistance in evaluation of P-glycoprotein (p170); and Dr. M. Narachi of Amgen, Inc., for supplying recombinant IFN-α and useful discussions.

REFERENCES

1. Dayal, H., and Kinman, J. Epidemiology of kidney cancer. Semin. Oncol., *10:* 366–377, 1983.
2. Silverberg, E., and Lubera, J. A. Cancer statistics, 1989. Cancer (Phila.), *39:* 3–20, 1989.
3. Ritchie, A. W., and Chisholm, G. D. The natural history of renal carcinoma. Semin. Oncol., *10:* 390–400, 1983.
4. Robson, C. J., Churchill, B. M., and Anderson, W. The results of radical nephrectomy for renal cell carcinoma. J. Urol., *101:* 297–301, 1969.
5. Quesada, J. R., Swanson, D. A., Trindade, A., and Gutterman, J. U. Renal cell carcinoma: antitumor effects of leukocyte interferon. Cancer Res., *43:* 940–947, 1983.
6. Quesada, J. R., Swanson, D. A., and Gutterman, J. U. Phase II study of interferon alpha in metastatic renal-cell carcinoma: a progress report. J. Clin. Oncol., *3:* 1086–1092, 1985.
7. Krown, S. E. Interferon treatment of renal cell carcinoma. Cancer (Phila.), *59:* 647–651, 1987.
8. Buzaid, A. C., and Todd, M. B. Therapeutic options in renal cell carcinoma. Semin. Oncol., *16:* 12–19, 1989.
9. Bander, N. H., Cordon Cardo, C., Finstad, C. L., *et al.* Immunohistologic dissection of the human kidney using monoclonal antibodies. J. Urol., *133:* 502–505, 1985.
10. Finstad, N. H., Cordon Cardo, C., Bander, N. H., Whitmore, W. F., Melamed, M. R., and Old, L. J. Specificity analysis of mouse monoclonal antibodies defining cell surface antigens of human renal cancer. Proc. Natl. Acad. Sci. USA, *82:* 2955–2959, 1985.
11. Cordon Cardo, C., Finstad, C. L., Bander, N. H., and Melamed, M. R. Immunoanatomic distribution of cytostructural and tissue-associated antigens in the human urinary tract. Am. J. Pathol., *126:* 269–284, 1987.
12. Bander, N. H. Monoclonal antibodies: state of the art. J. Urol., *137:* 603–612, 1987.
13. Bander, N. H., Finstad, C. L., Cordon-Cardo, C., *et al.* Analysis of a mouse monoclonal antibody that reacts with a specific region of the human proximal tubule and subsets of renal cell carcinomas. Cancer Res., *49:* 6774–6780, 1989.
14. Bander, N. H. Monoclonal antibodies in urologic oncology. Cancer (Phila.), *60:* 658–667, 1987.
15. Ueda, R., Shiku, H., Pfreundschuh, M., *et al.* Cell surface antigens of human renal cancer defined by autologous typing. J. Exp. Med., *150:* 564–579, 1979.
16. Albino, A. P., Houghton, A. N., Eisinger, M., *et al.* Class II histocompatibility antigen expression in human melanocytes transformed by Harvey murine sarcoma virus (Ha-MSV) and Kirsten MSV retroviruses. J. Exp. Med., *164:* 1710–1722, 1986.
17. Albino, A. P., Le Strange, R., Oliff, A. I., Furth, M. E., and Old, L. J. Transforming *ras* genes from human melanoma: a manifestation of tumour heterogeneity? Nature (Lond.), *308:* 69–72, 1984.
18. Neidhart, J. A., Schmidt, S., Rosenblum, M., *et al.* Phase 1 study of recombinant methionyl human consensus interferon (r-metHuIFN-Con1). J.

Downloaded from http://aacrjournals.org/cancerres/article-pdf/50/14/4190/2439840/cr0500144190.pdf by guest on 28 May 2024

Biol. Response Modifiers, *7:* 240–248, 1988.
19. Krown, S. E. Interferons and interferon inducers in cancer treatment. Semin. Oncol., *13:* 207–217, 1986.
20. Gutterman, J. U., Fine, S., Quesada, J., *et al.* Recombinant leukocyte A interferon: pharmacokinetics, single-dose tolerance, and biologic effects in cancer patients. Ann. Intern. Med., *96:* 549–556, 1982.
21. Bander, N. H. Study of the normal human kidney and kidney cancer with monoclonal antibodies. Uremia Invest., *8:* 263–273, 1985.
22. Pastan, I., and Gottesman, M. Multiple-drug resistance in human cancer. N. Engl. J. Med., *316:* 1388–1393, 1987.
23. Fojo, A. T., Shen, D. W., Mickley, L. A., Pastan, I., and Gottesman, M. M. Intrinsic drug resistance in human kidney cancer is associated with expression of a human multidrug-resistance gene. J. Clin. Oncol., *5:* 1922–1927, 1987.
24. Kanamaru, H., Kakehi, Y., Yoshida, O., Nakanishi, S., Pastan, I., and Gottesman, M. M. MDR1 RNA levels in human renal cell carcinomas: correlation with grade and prediction of reversal of doxorubicin resistance by quinidine in tumor epxlants. J. Natl. Cancer Inst., *81:* 844–849, 1989.
25. Thiebaut, F., Tsuruo, T., Hamada, H., Gottesman, M. M., Pastan, I., and Willingham, M. C. Cellular localization of the multidrug-resistance gene product P-glycoprotein in normal human tissues. Proc. Natl. Acad. Sci. USA, *84:* 7735–7738, 1987.
26. Fradet, Y., Cordon-Cardo, C., Thompson, T., *et al.* Cell surface antigens of human bladder cancer defined by mouse monoclonal antibodies. Proc. Natl. Acad. Sci. USA, *81:* 224–228, 1984.

Downloaded from http://aacrjournals.org/cancerres/article-pdf/50/14/4190/2439840/cr0500144190.pdf by guest on 28 May 2024