Up-Regulation of Retinoic Acid Receptor β Expression in Renal Cancers in Vivo Correlates with Response to 13-cis-Retinoic Acid and Interferon-α-2a


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ABSTRACT

Retinoic acid receptor-β (RAR-β) mRNA is not expressed by retinoid-resistant renal cancer cell lines but is present in retinoid-sensitive SK-RC-06 renal cancer cells and increases following incubation with retinoic acid (RA), suggesting that the antitumor action of RA is mediated through RAR-β (A. D. Hoffman et al., Clin. Cancer Res., 2: 1077–1082, 2996). To determine whether RAR-β expression correlates in vivo with major clinical response to patients with renal cell carcinoma (RCC) who were treated with retinoid-based therapy, we used in situ hybridization to analyze RAR-β expression in tumor specimens obtained from patients who were treated on a clinical trial with 13-cis-RA and IFN-α. Thirty-three tissue specimens were analyzed (23 pretreatment and 10 on-treatment). mRNA expression was based on staining intensity, with scores within tumor cells ranging from 0 to 2, where a score of 0 indicated absence of staining, a score of 1 indicated weak staining, and a score of 2 indicated strong staining. RAR-β expression was present in 22 of 23 (96%) pretreatment and 9 of 10 (90%) on-treatment specimens. Pretreatment levels of expression did not associate with the site of biopsy and did not predict for major clinical response to RA plus IFN-α therapy (two-sided Fisher’s exact test, P = 0.826). However, an increase in the intensity of RAR-β mRNA expression was detected in four of five (80%) patients who achieved a major response but in none of the five patients with progressive disease in whom sequential biopsies were available (two-sided Fisher’s exact test, P = 0.048). These data show that RAR-β transcripts increase in tumor cells of RCC patients who clinically respond to retinoid-based therapy. Retinoids that potently induce RAR-β expression should be evaluated in the treatment of advanced RCC.

INTRODUCTION

RCC is a frequent cause of cancer morbidity and mortality, with over 10,000 deaths per year in the United States (1). This fact reflects a lack of effective chemotherapeutic or biological treatment modalities for patients with renal cancer who develop metastatic disease. We reported that the combination of 13-CRA and IFN-α for the treatment of patients with advanced RCC resulted in a major response proportion of 30% (2), which was appreciatively better than that reported for previous clinical trials with IFN-α alone at Memorial Sloan-Kettering Cancer Center (3). These data suggested that RA potentiates the antitumor action of IFN. We assessed the growth-inhibitory effects of RA on renal cancer cells by conducting a clinical trial with 13-CRA alone in 25 patients with advanced RCC (4) and by examining the antiproliferative effects of 13-CRA on 12 RCC cell lines (5). We observed that there were no major responses in renal cancer patients treated with 13-CRA and that proliferation of 11 of 12 renal cancer cell lines was not appreciably inhibited by 13-CRA. Only the growth of SK-RC-06 cells was significantly (>90%) inhibited.

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Table 1  RAR-β mRNA expression of renal cancer specimens prior to treatment

<table>
<thead>
<tr>
<th>Expression intensity score</th>
<th>No. of patients who achieved a major response</th>
<th>No. of patients who did not achieve a major response</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>6</td>
</tr>
</tbody>
</table>

* Two-sided Fisher’s exact test, P = 0.826.
RARs mediate RA biological activity. Expression of a specific retinoid receptor mRNA associates with RA induced growth suppression in a variety of malignant cell lines (6–9). To determine whether a similar correlation occurred in RCCs, we examined the basal and induced expression of RAR-α, -β, and -γ in 12 renal cancer cell lines (5). Expression of RAR-α transcripts was abundant in all 12 cell lines examined, whereas low levels of RAR-γ transcripts were detectable in 6 of 10 renal cancers. Expression of RAR-α and -γ was not affected by 13-CRA. In contrast, RAR-β expression could not be detected or induced by 13-CRA treatment in all 11 retinoid-resistant cell lines. However, retinoid-sensitive SK-RC-06 cells basally expressed RAR-β transcripts, and RAR-β mRNA expression was up-regulated by 13-CRA treatment. Taken together, these data suggest that most RCCs are resistant to the inhibitory action of 13-CRA alone, that retinoid resistance is a cophenotype with absent RAR-β mRNA expression, and that the antiproliferative effect of 13-CRA on renal cancer cells correlates with basally expressed and 13-CRA-induced RAR-β expression.

These studies also implicate RAR-β as a mediator of RA action in RCC cells and suggest that RAR-β expression may serve as a marker to predict clinical responsiveness to retinoid-based treatment in patients. A previous study demonstrated that there was an increase in the level of RAR-β mRNA in oral leukoplakia lesions in patients treated with 13-CRA and that this increase showed a significant relationship to clinical response (10). To determine whether RAR-β mRNA expression in RCC cells in vivo predicts for response to retinoid-based therapy, we analyzed pre- and post-treatment RCC tissue specimens from selected RCC patients treated on a clinical trial of 13-CRA and IFN-α and correlated the results with clinical response. We report that up-regulation of RAR-β mRNA expression in RCC cells determined by in situ hybridization following treatment with 13-CRA and IFN-α significantly correlates with major response to this therapy.

MATERIALS AND METHODS

Treatment and Response Assessment. The results of the Phase II trial have been reported previously (2). Forty-five patients with advanced RCC gave informed consent and were treated with both 13-CRA (Accutane) and IFN (Roferon-A) obtained from Hoffman-LaRoche, Inc. (Nutley, NJ) through the Division of Cancer Treatment, National Cancer Institute (Bethesda, MD). 13-CRA was given p.o. at a dose of 1 mg/kg/day, divided between two doses each day. IFN was administered by a daily s.c. injection starting at 3 million units; dose escalation proceeded weekly to 6 million units and then 9 million units. Standard response criteria were used (2). Major responses included both complete and partial responses and were defined by a >50% decrease in the summed products of the perpendicular diameters of all measured lesions, for a minimum of 4 weeks. Complete responses were noted by the disappearance of all clinically evident disease, whereas the remaining major responses were classified as partial responses. The duration of a response was determined from the start of treatment.

Renal Cancer Tissue Specimens. Renal cancer specimens were obtained from 23 patients on the clinical trial. Pretreatment specimens included nephrectomy specimens or biopsy specimens obtained at Memorial Sloan-Kettering Cancer Center. Tissue was immediately brought to the pathology department, fixed in formalin, and embedded in paraffin. H&E staining was used to confirm malignant phenotype of the specimens. Ten on-treatment specimens were obtained from patients on active therapy or within 1 month of discontinuing 13-CRA and IFN.

In Situ Hybridization. RAR-β mRNA expression was determined using nonradioactive in situ hybridization without modifications, as described previously (11). Confirmation of the quality and specificity of the digoxigenin-labeled probe was verified by Northern blotting, as described previously (11). Adjacent tissue sections were stained with H&E to confirm the presence of tumor cells. Stained sections were reviewed under a Nikon microscope by three researchers, including one pathologist, who were unaware of the clinical status of the patients from whom the specimens had been obtained. RAR-β mRNA expression was based on staining intensity scores within tumor cells ranging from 0 to 2, where a score of 0 indicated absence of staining, a score of 1 indicated weak staining, and a score of 2 indicated strong staining. Most specimens stained uniformly. If staining was inhomogeneous, the predominant pattern was scored. Staining of an identical normal human kidney tissue section was performed with each experiment and used to confirm consistent levels of staining intensity. Change in relative staining intensity was classified as up-regulated if the staining intensity score increased, unchanged if it remained the same, and down-regulated if the score decreased.

Statistical Analysis. Subjects were categorized by pre-treatment level of RAR-β mRNA expression and change in relative staining intensity of RAR-β mRNA expression on treatment. The relationship between expression and treatment response was assessed using the two-sided Fisher’s exact test (12, 13).

RESULTS

RAR-β mRNA Expression. Formalin-fixed, paraffin-embedded RCC tissue specimens obtained by surgical resection or biopsy prior to treatment were available from 23 patients,
Table 2 Comparison of RAR-β mRNA expression in pre- and on-treatment renal tumor specimens

<table>
<thead>
<tr>
<th>Patient</th>
<th>Histology</th>
<th>Pretreatment specimen site</th>
<th>Staining intensity</th>
<th>On-treatment specimen site</th>
<th>Staining intensity</th>
<th>5 in RAR-β intensity</th>
</tr>
</thead>
</table>
| A. Patients who achieved a major clinical response
| 1       | Clear cell | Kidney                    | 0                 | Lung                      | 1                 | Increased            |
| 2       | Clear cell | Nephrectomy               | 1                 | Lung                      | 2                 | Increased            |
| 3       | Papillary  | Kidney                    | 1                 | Lymph node                | 2                 | Increased            |
| 4       | Clear cell | Soft tissue               | 1                 | Soft tissue               | 2                 | Increased            |
| 5       | Clear cell | Lung                      | 2                 | Lung and mediastinal lymph node | 2         | No change            |
| B. Patients who did not achieve a major clinical response
| 6       | Clear cell | Kidney                    | 1                 | Bone                      | 0                 | Decreased            |
| 7       | Clear cell | Kidney                    | 2                 | Bone                      | 1                 | Decreased            |
| 8       | Papillary  | Nephrectomy               | 2                 | Colon                     | 2                 | No change            |
| 9       | Clear cell | Kidney                    | 2                 | Nephrectomy               | 2                 | No change            |
| 10      | Clear cell | Nephrectomy               | 2                 | Soft tissue               | 1                 | Decreased            |

A Two-sided Fisher’s exact test, P = 0.048.
B All five patients achieved partial responses.

including 9 nephrectomy specimens and 14 biopsies (6 renal tumors, 4 pulmonary nodules, 3 bone, and 1 soft tissue). Ten of these 23 patients also had RCC tissue procured during treatment or within 4 weeks of discontinuing therapy.

RAR-β mRNA expression could be detected in 22 of 23 (96%) of pretreatment RCC specimens. Pretreatment RAR-β mRNA expression intensities (scored 0, 1, or 2) were as follows: score 0, 1 patient (4%); score 1, 13 patients (57%); and score 2, 9 patients (39%). Pretreatment levels of expression did not associate with the site of biopsy and did not predict for major clinical response to 13-CRA plus IFN therapy (two-sided Fisher’s exact test, P = 0.826; Table 1).

Expression analysis of the 10 available on-treatment specimens showed one patient (10%) with a score of 0, three patients (30%) with a score of 1, and six patients (60%) with a score of 2. Comparison of the change in staining intensity in tumor specimens from the same patient was determined and categorized according to clinical response to therapy (Table 2; Fig. 1). An increase in the intensity of RAR-β mRNA expression was detected in four of five (80%) patients who achieved a major response but in none of the five patients with progressive disease in whom sequential biopsies were available (two-sided Fisher’s exact test, P = 0.048). The fifth patient who achieved a major response exhibited strong RAR-β staining intensity (score = 2) in tumor cells prior to treatment, which increased with therapy (Fig. 1D); however, the staining intensity after the start of treatment could not be scored higher than 2, and the expression was considered unchanged for the statistical analysis.

DISCUSSION

In contrast to most renal cancer cell lines, which do not express detectable transcripts for RAR-β, >90% of renal tumors in vivo express basal levels of RAR-β mRNA, as determined by in situ hybridization with a RAR-β-specific probe. Furthermore, expression intensity could not predict for a major clinical response to treatment with 13-CRA and IFN, indicating that pretreatment RAR-β staining cannot be used as a marker to select retinoid-based therapies for patients with advanced RCC. However, up-regulation of RAR-β mRNA following 13-CRA and IFN therapy was associated with major clinical responses. Patients whose tumor cells increased their expression of RAR-β experienced tumor regression, whereas patients whose tumor cells did not increase RAR-β expression progressed on therapy. These in vivo data confirm our in vitro study of retinoid-sensitive SK-RC-06 renal cancer cells, which also up-regulate RAR-β expression, concordant with a >90% growth inhibition (5). Both of these studies support the concept that the antitumor effect of RA in RCC cells is mediated through RAR-β.

The results of this study are similar to those observed in patients with oral leukoplasia lesions who were treated with 13-CRA, in whom increases in the level of RAR-β mRNA showed a significant relationship to clinical response (10). In that study, sequential biopsies were obtained from near or identical regions of the oral cavity, allowing a direct comparison of pre- and post-treatment samples. Our study is limited by our inability to obtain sequential specimens from the same tissue site and by the comparison of pre- and on-therapy RAR-β expression between different tissues from the same patient. Nevertheless, despite the availability of sequential biopsies from only 10 patients, we observed a statistical difference in the increase in RAR-β mRNA intensity between responding and progressing patients. Therapy with 13-CRA and IFN-α is systemic; therefore, in theory, any effect of the retinoid on RAR-β transcripts in RCC cells should occur at multiple sites of metastases.

The patients in this study were treated with IFN-α in addition to 13-CRA. The mechanism of cooperation between 13-CRA and IFN in RCC is unknown (14–17). Recent studies in breast and cervical cancer suggest that RA can modulate IFN-inducible gene expression by augmenting the transcription of IFN-stimulated genes (17). In squamous cell carcinomas, RA and IFN treatment induces the transcription of IFN regulatory factor 1, concomitant with an induction of apoptosis (17). Although we have shown that combining 13-CRA and IFN-α results in a significant increase in growth inhibition in RCC cell lines compared to 13-CRA or IFN-α alone, we could not demonstrate any effect of 13-CRA on IFN-induced transcription of IFN-responsive genes by exam-
ining the binding of transacting factors to an IFN-stimulated response element.\(^4\)

In summary, retinoids can exert a significant effect on growth and differentiation of both normal and neoplastic cells (18, 19). These effects are mediated through retinoid receptors. Our preclinical and clinical studies suggest that retinoid action in RCC cells is mediated through the RAR-β isoform. Moreover, our data imply that retinoids that potently induce RAR-β expression should be evaluated in the treatment of advanced RCC.

**REFERENCES**


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