Interleukin-13 Receptor-targeted Cancer Therapy in an Immunodeficient Animal Model of Human Head and Neck Cancer

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ABSTRACT

Although interleukin-13 receptors (IL-13R) are overexpressed on several head and neck cancer cell lines, a majority of cell lines express only low levels of IL-13. We have found that the presence of an interleukin-13-binding protein IL-13Rα2 chain plays an important role in ligand binding and internalization. We showed that the gene transfer of IL-13Rα2 chain into various solid tumor cell lines that express few IL-13Rs can dramatically sensitize cells to the cytotoxic effect of a recombinant chimeric protein composed of interleukin-13 and a mutated form of Pseudomonas exotoxin A, IL-13/PE38QQR. Based on the expression of IL-13R, we have classified five head and neck cancer cell lines into two groups: (a) IL-13Rα2 chain-positive cell lines (SCC-25 and KCCT873); and (b) IL-13Rα2 chain-negative cell lines (A253, YCUT891, and KCCT871). By plasmid-mediated stable gene transfer, we demonstrate that not only IL-13Rα2 chain-positive head and neck cancer cell lines but also IL-13Rα2 chain-negative cell lines can dramatically increase sensitivity to IL-13 toxin by 520-1000-fold compared with mock-transfected control cells after genetic alteration to express high levels of the IL-13Rα2 chain. In animal studies, i.p. or intratumoral administration of IL-13/PE38QQR given daily or on alternate days for 3–5 days showed dramatic tumor response with complete remission in intratumorally injected tumors in both IL-13Rα2 chain-positive and -negative but transfected with IL-13Rα2 chain head and neck tumor implanted s.c. in nude mice. These results demonstrate that by using a combination approach of gene transfer and systemic or locoregional cytotoxin therapy, the IL-13R represents a new potent target for head and neck cancer therapy.

INTRODUCTION

Although advances in diagnosis and combined modality therapy have improved functional outcome, the incidence and mortality rate from SCCHN in the United States has not improved significantly in the past 20 years (1, 2). To address this problem and to generate cancer-targeted novel therapeutic agents, over the past decade we have opted to identify expression of unique cell surface receptors on solid tumor cells and primary cell cultures. About 5 years ago, we identified plasma membrane receptors for Th-2-derived cytokine IL-13 on several human renal cell carcinoma cell lines (3). Since then, we have reported that a variety of human solid cancer cell lines express IL-13R (4–10). IL-13 plays a major role in inflammatory diseases and may play a prominent role in cancer because receptors for this cytokine are overexpressed, and IL-13 is an autocrine growth factor for some cancer cells (11, 12).

In recent years, we have examined the structure of IL-13R in various cell types (5, 9, 13–16). We have reported that IL-13 binds to two isoforms of M, 65,000 proteins in human renal cell carcinoma cells and that one of these proteins also binds IL-4 (3). On the basis of the binding characteristics, cross-linking, displacement of radiolabeled IL-4 and IL-13, and interaction with other receptors in various cell types, we hypothesized that IL-13R may exist as three different types (9, 13–16). Two different chains (IL-13Rα1 and IL-13Rα2, also known as IL-13Rα2 and IL-13Rα, respectively) of the IL-13R system have been cloned, which correspond to two of the M, 65,000 isoforms, as we originally proposed (3). The murine and human IL-13Rα1 chains were cloned first (17, 18). This chain binds IL-13 at a low level, but when coupled with primary IL-4-binding protein IL-4Rβ chain (also known as IL-4Rβ2), it binds IL-13 and mediates IL-13-induced signaling (19). The second chain of IL-13R, termed IL-13Rα2, has also been cloned from a human renal cell carcinoma cell line (Caki-1). This chain has a short intracellular domain and binds IL-13 with high affinity (20).

Recently, we have demonstrated that the primary IL-13-binding protein, IL-13Rα2 chain, plays an important role in IL-13 binding and internalization (21). This chain is reported to be expressed on a variety of cancer cell lines; however, some cancer types do not express this receptor chain or express a low level of this receptor chain. Because of the low-level expression of IL-13Rα2 chain, these cells show modest sensitivity to an IL-13R-targeted cytotoxin, IL-13/PE38QQR, which is composed of IL-13 and a mutated form of Pseudomonas exotoxin A (4, 6–8, 10). Based on our hypothesis that gene transfer of this chain into cancer cells might increase their sensitivity to IL-13 toxin, we demonstrated that transient transfection of this chain into cancer cell lines expressing low levels of IL-13Rα2 chain or no IL-13Rα2 chain increased their sensitivity to IL-13 toxin in vitro (22). Because only 20% of SCCHN cell lines express high levels of IL-13R, we classified SCCHN cell lines into two groups: (a) cell lines that express IL-13Rα2 chain (SCC-25 and KCCT873); and (b) cell lines with no or low expression of IL-13Rα2 chain (A253, YCUT891, and KCCT871). By generating IL-13Rα2 stable transfecants, we demonstrate the proof of principle that not only IL-13Rα2 chain-positive SCCHN cell lines but also IL-13Rα2-negative cell lines can be dramatically sensitized to the antitumor activity of IL-13 toxin after genetic alteration to express high levels of IL-13Rα2 chain in vitro and in vivo.

MATERIALS AND METHODS

Recombinant Cytokine and Toxin. Recombinant human IL-4 and IL-13 were produced and purified to homogeneity in our laboratory (23). Recombinant IL-13/PE38QQR was also produced and purified in our laboratory (4, 24).

Cell Lines. Human head and neck cancer cell lines (SCC-25 and A253) were purchased from the American Type Culture Collection (Manassas, VA). KCCT873, YCUT891, and KCCT871 cell lines were established in the Department of Otolaryngology, Yokohama City University School of Medicine or Research Institute, Kanagawa Cancer Center (Yokohama, Japan; Ref. 25). Cells were cultured in DMEM/Ham’s F-12 (SCC-25), McCoy’s 5A medium (A253), or RPMI 1640 (all other cell lines) containing 10% fetal bovine serum (Biowhittaker Inc., Walkersville, MD), 1 mM HEPES, 1 mM l-glutamine, 100

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3 These studies were conducted as part of a collaboration between the FDA and Neo Pharm Inc. under a Cooperative Research and Development Agreement (CRADA).

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5 The abbreviations used are: SCCHN, squamous cell carcinoma of the head and neck; IL, interleukin; IL-13, interleukin-13 receptor; IL-4R, interleukin-4 receptor; γc, common γ-chain; RT, reverse transcription; HSA, human serum albumin; i.t., intratumoral.
μg/ml penicillin, 100 μg/ml streptomycin (Biowhittaker Inc.), and 400 ng/ml hydrocortisone (hydrocortisone was only added to medium for SCC-25, Sigma Chemical Co., St. Louis, MO).

Stable Transfection and Selection. cDNA encoding human IL-13Rα2 chain (20) was cloned into pME18S mammalian expression vector (26). Plasmid DNA (12 μg/100-mm culture dish) was cotransfected with 1.2 μg of pPUR selection vector (Clontech Laboratories, Inc., Palo Alto, CA) into semiclonal cells using GenePORTER transfection reagent (Gene Therapy Systems, San Diego, CA) according to the manufacturer’s instructions. Briefly, cells (2 × 10⁶ cells/100-mm dish) were incubated with the DNA-GenePORTER mixture for 5 h in DMEM (Biowhittaker Inc.). DMEM containing deoxynucleotide triphosphate, 1 unit/tumor was then added, and incubation was continued. Twenty-four h after transfection, the medium was changed to DMEM with 10% FBS, and cells were incubated for an additional 24 h. At 48 h after the start of transfection, cells were trypsinized and cultured in selection medium containing 1 μg/ml puromycin (Clontech Laboratories, Inc.). Cells were maintained for 4 weeks in the same medium, which was replaced every 3 days. Resistant clones (25 A253 clones, 13 YCUT891 clones, and 5 KCCCT871 clones) isolated with the cloning cylinder (Bel-Art Products, Pequannock, NJ) were characterized for IL-13Rα2 chain expression by RT-PCR and radioiodide receptor binding assays. Finally, one of each of the IL-13Rα2-overexpressing clones (termed A253α2, YCUT891α2, and KCCCT871α2) were selected for further analysis. The vector control (mock)-transfected cell lines A253mc, YCUT891mc, and KCCCT871mc were used for comparison with IL-13Rα2-transfected cells. To reduce antibiotic side effects, puromycin was removed at least 14 days before the experiments were performed.

RT-PCR Analysis. To detect the mRNA expression of IL-13R chains in SCCHN cells, total RNA was isolated using Trizol reagent (Life Technologies, Inc., Grand Island, NY), and then RT-PCR analysis was performed. Two μg of total RNA were incubated for 30 min at 42°C in 20 μl of reaction buffer containing 10 mM Tris-HCl (pH 8.3), 5 mM MgCl₂, 50 mM KCl, 1 mM each deoxynucleotide triphosphate, 1 unit/μl RNA inhibitor, 2.5 μM random hexamer, and 2.5 units/μl Moloney murine leukemia virus reverse transcriptase (Perkin-Elmer Corp., Norwalk, CT). A 10-μl aliquot of the RT reaction was amplified in a 100-μl (final volume) PCR mixture containing 10 mM Tris-HCl (pH 8.3), 2 mM MgCl₂, 50 mM KCl, 1 unit of AmpliTaq Gold DNA polymerase (Perkin-Elmer Corp.), and 0.1 μg of specific primers for IL-13Rα2, IL-13Rα1, IL-4Rα, or γchains (27). The PCR product (30 μl) was run on a 2% agarose gel for UV analysis.

Radioiodide Receptor Binding Assays. Recombinant human IL-13 or IL-4 was labeled with 125I (Amersham Corp., Arlington Heights, IL) using Iodo-Gen reagent (Pierce, Rockford, IL) as described previously (28). The specific activity of the radiolabeled cytokines was estimated to be 6.0 μCi/μg protein (IL-13) or 28 μCi/μg protein (IL-4). For binding experiments, 5 × 10⁵ cells in 100 μl of incubation buffer (RPMI 1640 containing 0.2% HSA and 10 mM HEPES) were incubated with 200 pm 125I-IL-13 or 125I-IL-4 with or without 40 nM unlabeled IL-4 or IL-13 at 4°C for 2 h. Cell-bound radiolabeled cytokine was separated from unbound cytokine by centrifugation through a phthalate oil gradient, and radioactivity was determined with a gamma counter (Wallac, Gaithersburg, MD). The number of binding sites/cell was calculated based on the specific binding of radiolabeled cytokine as described previously (22).

Protein Synthesis Inhibition Assay. The cytotoxic activity of IL-13 toxin was tested as described previously (29). Typically, 10⁶ cells were cultured in leuine-free medium with or without various concentrations of IL-13-PE38QQR for 20–22 h at 37°C, and then 1 μCi of [3H]leucine (New England Nuclear Research Products, Boston, MA) was added to each well and incubated for an additional 4 h. Cells were harvested, and radioactivity incorporated into cells was measured by a beta plate counter (Wallac).

Animals. Four-week-old athymic nude mice (body weight, about 20 g) were obtained from Frederick Cancer Center Animal Facilities (National Cancer Institute, Frederick, MD). The mice were housed in filter-top cages in a laminar flow hood under pathogen-free conditions with 12-h light/12-h dark cycles. Animal care was in accordance with the guidelines of the NIH Animal Research Advisory Committee.

Human Head and Neck Cancer Xenografts and Treatment. Human head and neck tumors were established in nude mice by s.c. injection of 5 × 10⁶ SCC-25, KCCCT873, A253mc, A253α2, YCUT891mc, or YCUT891α2 cells in 150 μl of PBS plus 0.2% HSA into the flank. Palpable tumors developed within 3–4 days. The mice then received injections of exipient (0.2% HSA in PBS) or chimeric toxin either i.p. (500 μl) or i.t. (30 μl) using a 27-gauge needle.

Statistical Analysis. Tumor sizes were calculated by multiplying the length and width of the tumor on a given day. The statistical significance of tumor regression was calculated by Student’s t test.

RESULTS

Subunit Structure of IL-13R on Head and Neck Cancer Cells. Five SCCHN cell lines were examined for the expression of mRNA for various putative IL-13R subunits (IL-13Rα2, IL-13Rα1, IL-4Rα, and γchains) by RT-PCR. As shown in Fig. 1, we found that mRNA for IL-13Rα1 and IL-4Rα chains was present in all of the cell lines examined. However, no SCCHN cell lines showed the presence of γ chain mRNA. Very low level expression or no expression of IL-13Rα2 chain was observed in A253mc, YCUT891mc, and KCCCT871mc cells. As expected, IL-13Rα2-transfected cell lines (A253α2, YCUT891α2, and KCCCT871α2) showed ample mRNA expression. PM-RCC cells that express IL-13Rα2, IL-13Rα1, and IL-4Rα chains and H9 T lymphoma cells that express γ chain mRNA served as positive controls.

IL-13 Binding to IL-13Rα2 Chain-positive and -negative SCCHN Cell Lines. We then determined the expression and binding affinity of IL-13R on SCCHN cell lines by 125I-IL-13 binding assays. Two IL-13Rα2 chain-positive cell lines and three IL-13Rα2 chain-negative cell lines and transfectants were labeled with 125I-IL-13 in the absence or presence of a 200-fold molar excess of IL-13. As shown in Fig. 2A, 125I-IL-13 bound to SCCHN cells at almost same degree, and an excess of unlabeled IL-13 displaced the binding of 125I-IL-13. Because IL-13R and IL-4R share two chains, we also examined whether IL-4 can also displace the IL-13 binding in SCCHN cells (16, 25, 26). As shown in Fig. 2A, IL-4 also displaced 125I-IL-13 binding in KCCCT873 cells; however, in SCC-25 cells, IL-4 showed only minimal displacement of 125I-IL-13 binding.

The three SCCHN cell lines that have no IL-13 binding component (IL-13Rα2 chain) showed very low binding to 125I-IL-13 (Fig. 2B). However, when these cells were transfected with IL-13Rα2 chain, the binding activity of 125I-IL-13 was dramatically increased. An excess of unlabeled IL-13 inhibited the binding of 125I-IL-13, indicating specificizity. Interestingly, unlabeled IL-4 showed minimal displacement of 125I-IL-13 binding in YCUT891 and KCCCT871 cell lines. On the other hand, IL-4 partially displaced 125I-IL-13 binding in the A253 cell line. Because SCCHN cells line express IL-4R, we also deter-
mined the IL-4 binding sites in these cells (25). From these experiments, we calculated the number of IL-13-binding sites on IL-13Rα2 chain-positive and -negative cell lines. As shown in Table 1, in IL-13Rα2 chain-negative cell lines, after transfection of IL-13Rα2 chain, the number of IL-13-binding sites increased 48–850-fold compared with that of control cells. However, the number of IL-4 binding sites did not increase in IL-13Rα2-transfected cells, except in A253 cells, which showed a slight increase in the number of IL-4-binding sites.

SCCHN Cells Transfected with IL-13Rα2 Chain Show Increased Sensitivity to IL-13-PE38QQR. We have produced a chimeric protein composed of IL-13 and a truncated form of Pseudomonas exotoxin A (IL-13-PE38QQR), which was found to be potently cytotoxic to IL-13R-positive solid tumor cells (4, 7, 9). To determine whether IL-13Rα2 chain-positive SCCHN cell lines are sensitive to IL-13 toxin, we evaluated the cytotoxicity of this molecule to SCC-25 and KCCT873 cells. As shown in Fig. 3A, IL-13 toxin was cytotoxic to these cell lines, and the IC50 (the protein concentration required for the inhibition of protein synthesis by 50%) was 2.4 and 4.0 ng/ml, respectively (Table 1). The cytotoxic activity of IL-13-PE38QQR was neutralized by excess IL-13 and partially neutralized by IL-4 only in the KCCT873 cell line.

In cells that express very little or no IL-13Rα2 chain, IL-13 toxin is minimally cytotoxic. Therefore, to explore whether introduction of this chain into the cells increases the sensitivity of IL-13 toxin, we used stable transfectants of this chain. As shown in Fig. 3B, transfection of the IL-13Rα2 chain improved the sensitivity of all three cell lines to the cytotoxic effect of IL-13 toxin. IC50 values in the three cell lines improved from 520-fold to 1000-fold compared with control cells (Table 1). The increase in sensitivity to IL-13 toxin correlated with the increase in IL-13R-binding sites. The cytotoxic activity of IL-13 toxin in IL-13Rα2-transfected cells was blocked by an excess of IL-13 in all three cell lines, indicating that cytotoxicity mediated by this molecule is specific. Similar to binding data, IL-4 partially inhibited the cytotoxic activity of IL-13 toxin in the A253α2 cell line.

The i.p. Antitumor Activity of IL-13 Toxin to IL-13Rα2 Chain-positive SCCHN Tumors. To explore IL-13 toxin-mediated antitumor activity in IL-13Rα2 chain-positive SCCHN cell lines, we injected nude mice with SCC-25 or KCCT873 tumor cells i.p. with IL-13-PE38QQR twice daily for 5 days from day 4 to day 8 (a total of 10 injections). As shown in Fig. 4A, all SCC-25 tumors started regressing during the treatment, and one tumor completely disappeared by day 8. Although one tumor began to appear on day 11, by day 43 the mean size of the tumors remained small, similar to the size of tumors on the day of the first injection (23 mm²). By day 75, treated tumors gradually grew to 35 mm², and the reduction in tumor size was 74% (P < 0.001) compared with control tumors (137 mm²).

In the KCCT873 tumor model, all tumors started regressing during the treatment, and by day 8, tumors decreased to very small masses (Fig. 4B; 7 mm³). Thereafter, the tumors started growing gradually; however, the size remained significantly smaller compared with that of control tumors. Because tumors in control mice injected with vehicle only continued to grow exponentially, these mice were killed on day 36. The reduction in tumor size in the treated group on day 36 was 75% [46 mm² (P < 0.0006)] compared with tumors in control group (180 mm²).

The i.t. IL-13 Toxin Treatment Induced Total Eradication of IL-13Rα2 Chain-positive SCCHN Tumors. We also assessed the efficacy of i.t. administration of IL-13 toxin against SCC-25 and KCCT873 tumors. Treatment of SCC-25 tumors with i.t. IL-13-PE38QQR (250 μg/kg/day on alternate days for 3 days) inhibited tumor growth, and two of four tumors completely regressed by day 7 (Fig. 5A). By day 11, the growth of all treated tumors was arrested, then tumors subsequently disappeared completely. Although a palpable tumor appeared in one mouse on day 15, three mice remained tumor free until the day they were killed (day 90; data not shown).

As shown in Fig. 5B, treatment of KCCT873 tumors with i.t. IL-13 toxin (250 μg/kg/day on alternate days for 3 days) reduced tumor size, and one of four tumors showed complete regression by day 7. By day 11, one more tumor disappeared in the group of treated mice. On day 15, palpable tumors appeared in those mice, and all of the tumors began to grow gradually; however, the size of the tumor was significantly smaller, and the reduction in tumor size in the treated group on day 36 was 77% [41 mm² (P < 0.0008)] compared with tumors in the vehicle only-injected control group (180 mm²).

### Table 1 IL-4R- and IL-13R-binding sites on head and neck cancer cell lines and cytotoxicity of IL-13 toxin

<table>
<thead>
<tr>
<th>Cell line</th>
<th>No. of IL-4R-binding sites (sites/cell)</th>
<th>IC50 (ng/ml)</th>
</tr>
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<tbody>
<tr>
<td>SCC-25</td>
<td>13000 ± 510</td>
<td>2.4 ± 0.6</td>
</tr>
<tr>
<td>KCCT873</td>
<td>7600 ± 810</td>
<td>4.0 ± 0.5</td>
</tr>
<tr>
<td>A253mc</td>
<td>6100 ± 650</td>
<td>200 ± 50</td>
</tr>
<tr>
<td>A253α2</td>
<td>13000 ± 2800</td>
<td>0.2 ± 0.4</td>
</tr>
<tr>
<td>YCUT891mc</td>
<td>6300 ± 1200</td>
<td>520 ± 80</td>
</tr>
<tr>
<td>YCUT891α2</td>
<td>7100 ± 580</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>KCCT873mc</td>
<td>9100 ± 490</td>
<td>300 ± 85</td>
</tr>
<tr>
<td>KCCT873α2</td>
<td>8600 ± 60</td>
<td>0.3 ± 0.1</td>
</tr>
</tbody>
</table>

*IC50, the concentration of IL-13 toxin at which 50% inhibition of protein synthesis is observed compared with untreated cells.*
Sensitivity of IL-13Rα2 Chain-negative SCCHN Tumors to i.p. Administration of IL-13 Toxin Is Dramatically Increased by IL-13Rα2 Chain Gene Transfer. We found that transfection of the IL-13Rα2 chain improved the sensitivity of SCCHN cell lines to the cytotoxic effect of IL-13 toxin in vitro. To explore whether our findings can be applied to an in vivo tumor model, we injected nude mice with A253 or YCUT891 tumors i.p. with IL13-PE38QQR. As shown in Fig. 6A, in A253mc tumor-bearing mice, the tumors grew very well, and tumor treatment with IL-13 toxin (50 μg/kg) twice daily for 5 days (a total of 10 injections) did not result in a significant reduction in tumor size. On day 52, both treated mice and vehicle...
only-injected control group (187 mm²).

YCUT891mc and YCUT891 were only-injected control group (210 mm²).

transfected A253mc tumors, IL-13 toxin (50 g/kg; H9251) and YCUT891mc (H9253) or IL-13Rα2 chain cells A253mc (H9251) and YCUT891α2 (D) cells on day 0. The animals then received twice a day injections of IL-13 toxin (50 μg/kg; ○) or excipient only (□) for 5 days, as indicated by the arrows. YCUT891mc and YCUT891α2 tumor-bearing mice received a second course of injection from 25–29 days after implantation with same dose of IL-13 toxin as the first course. Each group had five animals; bars, SD.

only-injected mice were sacrificed, and the tumor size was 190 mm² and 168 mm², respectively.

Interestingly, on the other hand, although A253 tumors transfected with IL-13Rα2 chain (A253α2 tumors) grew as fast as vector only-transfected A253mc tumors, IL-13 toxin (50 μg/kg) treatment on the same schedule (twice daily for 5 days) resulted in significant antitumor activity. Two of five mice showed complete disappearance of their tumors by 4 days after the first injection (Fig. 6B). By day 24, the tumors of two more mice showed complete regression. These mice remained tumor free until day 52. Only one mouse had a very small tumor. On day 52, the reduction in tumor size in the treated group was 95% [10 mm² (P < 0.00002)] compared with tumors in vehicle only-injected control group (187 mm²).

YCUT891 tumor-bearing mice were also injected with IL-13-PE38QQR (50 μg/kg) twice daily for 5 days from day 4 to day 8. In addition, these mice also received a second course of treatment on day 25 through day 29. YCUT891mc tumors showed no sensitivity to IL-13 toxin on i.p. administration, even after the second course of the treatment (Fig. 6C). In contrast, after the first course of treatment with IL-13 toxin (50 μg/kg) from day 4 to day 8, YCUT891α2 tumors began to regress gradually (Fig. 6D). Although no tumor disappeared completely, the tumors remained smaller in size (about 24 mm²) compared with those of untreated mice. However, when mice were given the second course of IL-13 toxin (50 μg/kg) treatment from day 25 to day 29, the tumors began to regress again. By day 56, all of the tumor sizes remained small, similar to the size on the day of injection (33 mm²), and the reduction in tumor size in the treated group was 80% [41 mm² (P < 0.0001)] compared with tumors in the vehicle only-injected control group (210 mm²).

Complete Regression of IL-13Rα2 Chain-transfected SCCHN Tumors with IL-13 Toxin i.t. Treatment. To assess the efficacy of IL-13 toxin in IL-13Rα2-transfected tumors (A253α2 and YCUT891α2) mice were treated i.t. with IL-13-PE38QQR. In A253α2 tumor-bearing mice, after the treatment with IL-13 toxin (250 μg/kg/day on alternate days for 3 days from day 4), by day 7 tumors in two of five mice disappeared completely (Fig. 7A). By day 24, 100% of the tumors were completely regressed. All treated mice remained tumor free until day 52, when the experiment was terminated.

As shown in Fig. 7B, YCUT891α2 tumors were treated i.t. for two courses with IL-13 toxin (250 μg/kg/day on alternate days for 3 days) from day 4 to day 8 and from day 25 to day 29. After the first treatment course, tumors began to decrease in size; however, from day 14, tumors started growing again. No complete responders were observed at that time. After the second course of IL-13 toxin therapy, tumors began to regress again, and by day 28, three of five tumors disappeared completely. By day 49, two mice had developed a recurrence; however, one mouse remained tumor free until day 56. The reduction in tumor size in the treated group on day 56 was 86% [29 mm² (P < 0.00003)] compared with tumors in the vehicle only-injected control group (210 mm²).

DISCUSSION

In this study, we demonstrate the proof of principle that not only IL-13Rα2 chain-positive head and neck cancer cell lines but also IL-13Rα2 chain-negative cell lines can be dramatically sensitized to the antitumor activity of IL-13 toxin after gene transfer of the IL-13Rα2 chain. We classified SCCHN cell lines by the presence or absence of the IL-13Rα2 chain. Although RT-PCR analysis does not directly confirm the expression of IL-13R chains, our study implies that the IL-13R complex in SCCHN cell lines represents type I (where the IL-13Rα1 and IL-13Rα2 chains coexist on the cell surface) or type II (where the IL-13Rα1 and IL-4Rα chains form a complex) IL-13R. The common γc chain was not identified in these cells. The reason why some SCCHN cell lines express IL-13Rα2 chain is not known. Only 20% of 17 different SCCHN cell lines expressed the IL-13Rα2 chain. The significance of overexpression of the IL-13Rα2 chain is currently being investigated.

Interestingly, IL-4 was able to displace 125I-IL-13 binding to KCCT873 cells but not in SCC-25 cells. Furthermore, IL-4 was able to displace 125I-IL-13 in A253 cells transfected with the IL-13Rα2 chain, but not in YCUT891α2 and KCCT871α2 cells. These results...
are consistent with previous studies that have demonstrated that IL-4 can compete for the 125I-IL-13 binding sites on some cell lines but not on others (3, 8, 13, 14, 16, 18, 20). This interesting phenomenon may be explained by the stoichiometry of different receptor chain expression and usage. If cells constitutively express high levels of IL-4Rα chain, IL-4 will be able to displace both 125I-IL-13 binding and 125I-IL-4 binding. If the level of expression of this chain is lower, then IL-4 will not displace 125I-IL-13 binding. Our 125I-IL-4 binding studies partly support this conclusion. However, in SCC-25 cells that expressed a higher number of binding sites (13,000) than KCTT873 (7,600), IL-4 did not displace 125I-IL-13 binding. These results suggest that alternative mechanisms exist for this complex interaction between IL-4R and IL-13R.

It is of interest to note that both IL-13Rα2-positive and IL-13Rα2 stably transfected SCCHN cell lines showed high sensitivity to IL-13 toxin as assessed by cytotoxicity assays. However, SCCHN cells that did not express this chain were not sensitive. These data suggest that IL-13Rα2 chain is necessary for the internalization of enough molecules of Pseudomonas exotoxin for cytotoxicity to occur. We have also investigated the mechanism of cell death induced by IL-13 toxin. We observed that 30–40% of SCCHN cells die through apoptotic cell death by IL13-PE38QQR, whereas IL-13 alone had no effect.5

Consistent with in vitro sensitivity results, IL-13 toxin showed pronounced antitumor activity in vivo against tumors that expressed IL-13Rα2 chain naturally or artificially. In two tumor models, IL-13 toxin showed very high antitumor activity; however, when IL-13 toxin was administered i.p., no complete responders were observed. On i.t. administration, IL-13-PE produced complete responders in the SCC-25 tumor model, but not in the KCTT873 tumor model. On the other hand, IL-13Rα2 chain-negative tumors (A253mc and YCUT891mc) did not respond to IL-13 toxin at all by i.p. or i.t. routes even with two courses of IL-13 toxin treatment. However, when IL-13Rα2 chain-transfected tumor (A253α2)-bearing mice were injected i.p. with IL-13-PE38QQR, four of five mice showed complete disappearance of disease. Similarly, by the i.t. route, all animals showed complete regression of tumors. Interestingly, when IL-13Rα2 chain-transfected YCUT891 tumor (YCUT891α2)-bearing mice were injected with two courses of IL-13 toxin by i.p. or i.t. routes, none of these animals showed complete response. However, by both routes, a remarkable antitumor activity was observed. The mechanism of lack of complete responders in IL-13Rα2 chain-transfected YCUT891α2 tumors is not known. It is possible that IL-13Rα2 chain gene expression was not optimum. Although YCUT891α2 tumor cells expressed IL-13Rα2 chain mRNA, quantitative comparisons of IL-13α2 chain expression could not be performed. It is important to note that both A253α2 and YCUT891α2 cell lines expressed a similar density of IL-13R (Table 1). Thus, other mechanisms are operational in differential sensitivity to the IL-13 toxin in two tumor models. The efficiency of distribution of IL-13 toxin in the tumor bed may be another mechanism of this difference.

This is the first demonstration in which SCCHN cells that express low levels of IL-13R and have modest sensitivity to IL-13-targeted cytotoxins can enhance their sensitivities dramatically in vitro and in vivo after genetic transfer of only one chain of cytokine receptor. Because IL13-PE38QQR was found to be cytotoxic only to cancer cells that express IL-13R and not to human T and B cells, monocytes, normal endothelial cells, and resting or growth factor-activated bone marrow cells (6), our current findings offer promising possibilities for the utilization of IL-13 toxin for both IL-13Rα2 chain-positive and -negative SCCHN cancer therapy.

Although various strategies are being developed for immunotherapy or targeting of cancer, our current strategy is the only unique method that uses one cytokine receptor chain as a sensitizer to targeted cancer therapy. To further improve this strategy, we are currently examining the antitumor activity of IL13-PE38QQR after the direct in vivo gene transfer of the IL-13Rα2 chain into tumor in a SCCHN xenograft model (30, 31). In this approach, we are injecting plasmid DNA encoding IL-13Rα2 chain mixed with lipid directly into s.c. developed tumor in immunodeficient mice, followed by IL-13 toxin treatment by either systematic or i.t. administration. Because this approach could render unresponsive tumors sensitive to the IL-13 toxin in vivo, our strategy would be more beneficial for patients with SCCHN. Thus, we believe that our new strategy, which introduces a functional cytokine receptor into cancer cells, provides a novel useful technique combining gene therapy with cytotoxin therapy.

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