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**Review paper**

# Cytokine receptor as a sensitizer for targeted cancer therapy

**Koji Kawakami,[1] Mariko Kawakami[1] and Raj K Puri[1]**
[1]Laboratory of Molecular Tumor Biology, Division of Cellular and Gene Therapies, Center for Biologics Evaluation and Research, Food and Drug Administration, Bethesda, MD 20892, USA.

**Introducing a cytokine receptor as a sensitizer into cancer cells offers a unique opportunity for receptor-targeted cancer therapy. It has been shown that transfection of the tumor necrosis factor (TNF) receptor gene in cancer cells or exposing cancer cells to certain reagents which increase expression of TNF receptors results in enhancement of the cytotoxic effect of TNF. In addition, the literature suggests that Fas/CD95-mediated apoptotic tumor cell killing is augmented by gene transfer of Fas into cancer cells or treatment of cells with agents like cisplatin and interferon (IFN)-$\gamma$. In contrast to these approaches, we have discovered a new approach to cancer therapy; wherein introduction of a cytokine receptor chain into cancer cells sensitizes them to receptor-directed cytotoxins. We have demonstrated that when interleukin (IL)-13 receptor (IL-13R) $\alpha$2 chain, one of the two known IL-13 binding proteins, is introduced into cancer cells that do not express this chain the cells acquire extreme sensitivity to a chimeric fusion cytotoxin composed of IL-13 and a mutated form of *Pseudomonas* exotoxin (IL13-PE). Cells that do not express this chain or express low levels show limited sensitivity to IL13-PE. Acquisition of sensitivity to IL13-PE was observed both *in vitro* and *in vivo* when IL-13R$\alpha$2-transfected human tumor cells were implanted in immunodeficient animals followed by systemic or regional IL13-PE therapy. Our third generation experiments suggest that this approach is feasible for clinical situations as intratumor administration of plasmid carrying the IL-13R$\alpha$2 chain gene sensitized these tumors to systemic or regional IL13-PE therapy. This unique approach comprising gene transfer of cytokine receptor chain and receptor-targeted cytotoxin administration represents a novel strategy for cancer therapy. [© 2002 Lippincott Williams & Wilkins.]**

*Key words:* Cytotoxin therapy, Fas/CD95, gene transfer, immunotherapy, interleukin-13 receptor, sensitization.

Correspondence to RK Puri, Laboratory of Molecular Tumor Biology, Division of Cellular and Gene Therapies, Center for Biologics Evaluation and Research, Food and Drug Administration NIH Building 29B, Room 2NN10, 29 Lincoln Drive MSC 4555, Bethesda, MD 20892, USA.
Tel: (+1) 301 827-0471; Fax: (+1) 301 827-0449;
E-mail: puri@cber.fda.gov

## Introduction

Cancer cells are known to express specific antigens or cytokine receptors on their cell surface. Cell surface molecules like Fas/CD95 ligand can mediate growth inhibition signals by themselves. Because of that function, Fas/CD95-related molecules are called 'death receptors'. However, other cell surface receptors do not possess self-killing functions. Instead, these cell surface molecules can be targeted with monoclonal antibodies or targeted therapeutic agents. Thus, gene transfer or up-regulation of these target molecules can mediate inhibition of tumor growth or sensitize cancer cells to the antitumor effect of target-directed agents. A single cancer therapeutic approach is often less effective; addition of other approaches, e.g. 'sensitization' of cancer cells to the therapeutic moiety, is necessary for optimal anticancer therapy. In this article, we summarize what investigators have done in this context and describe a novel approach that combines cytokine receptor chain gene transfer and receptor-targeted cancer therapy, focusing on interleukin (IL)-13 receptor (IL-13R) $\alpha$2 chain gene transfer followed by IL-13 cytotoxin therapy.

## Cytokine receptor gene transfer in cancer immunotherapy

The first attempt to utilize cytokine receptor in cancer therapy was independent of targeted agent or death signaling. In this approach, cancer cells were transfected with a cytokine receptor gene and exposed to the naturally existing ligand, in the expectation of the cytokine would have antitumor activity. In this approach, Lin *et al.*

demonstrated that *in vitro* transfection of IL-2R$\gamma$ chain in head and neck cancer cells resulted in inhibition of tumor growth when treated with IL-2.[1] IL-2 has a limited antitumor activity in cancer *in vitro* and *in vivo*. IL-2R gene transfer in fibroblast cells was shown to maintain cellular binding of IL-2.[2–4]

The second approach in this category is gene transfer of a tumor-specific antigen receptor or antibody gene to immune cells that can specifically attack antigen-bearing cancer cells. It has been shown that T cell receptor gene transfected T cells or tumor-infiltrating lymphocytes were able to acquire tumor recognition ability.[5,6] Another potent immune effector, natural killer (NK) cell line (YT) was transfected with the human asialoglycoprotein receptor to show tumor cell killing through Thomsen–Friedenreich antigen.[7] Although these approaches demonstrated effective antitumor activities, the limitation of *in vitro* transfection efficiency in immune cells and trafficking of these cells to tumor targets *in vivo* may make it difficult to utilize these strategies successfully in the clinic.

Interferon (IFN)-$\gamma$ has potent antitumor activity to IFN-$\gamma$ receptor-expressing cancer cells. In an attempt to sensitize cancer cells to the effect of IFN-$\gamma$, Nishida *et al.* demonstrated that adenoviral-mediated transfection of IFN-$\gamma$ receptor $\alpha$ and $\beta$ chain genes in cancer cells resulted in *in vivo* inhibition of CT26 tumor growth after the systemic administration of IFN-$\gamma$.[8] This unique approach can be applied to any other cytokine receptor when its ligand has a natural growth modulatory effect.

Another approach in this category is gene transfer of the somatostatin receptor in cancer cells. Somatostatin is a 14-amino-acid peptide present mainly in the central nervous system and gastrointestinal tract that can inhibit tumor growth through its receptor (sst1–5) on tumor cells.[9] Rochaix *et al.* demonstrated that gene transfer of *sst2* in pancreatic cancer cells (BxPC-3) resulted in inhibition of tumor growth *in vivo*.[10] Gene transfer of *sst2* in tumor cells activated somatostatin production by the autocrine feedback loop, followed by *sst1* up-regulation in tumor cells, which also mediated the antiproliferative effect of somatostatin. This strategy is unique as *sst2*-transfected tumor cells can induce a bystander effect to distant untransfected tumor cells; however, further examination will be required to study the mechanism of action and assess its application in other types of cancer.

## Tumor necrosis factor (TNF) receptor gene transfer and sensitization of TNF-mediated cancer therapy

TNF is a cytokine mainly produced by activated macrophages, lymphoid cells, neutrophils, natural killer cells, keratinocytes and fibroblasts.[11] TNF has a potent cytotoxic effect on tumor cells and clinical studies have shown its potent usage with other chemotherapeutic agents.[12,13] The receptor for TNF is composed of 75-kDa (TNF-RII) and 55-kDa (TNF-RI) proteins, and the expression of these proteins widely varies between tumor cell types. To increase the cell lysis effect of TNF, TNF receptors were transfected in human lung and colon cancer cells, and the effect of TNF *in vitro* was found to be increased in these cells.[14,15] Antitumor activity of TNF and mutein TNF was also enhanced when pancreatic cancer cells (AsPC-1) transfected with TNF-RI were implanted into nude mice.[16]

Certain agents/methods, e.g. endothelial monocyte-activating polypeptide II (EMAP-II),[17] ionizing radiation,[18] lithium[19] and cisplatin[20], have been shown to increase the effect of TNF on tumor cells through TNF receptors. On the other hand, TNF receptor gene transfer approach is more focused on the direct association between ligand and receptor. Because the effect of TNF depends in part on intracellular signaling involving protein kinase C (PKC), regulation of PKC along with TNF receptor modulation and sensitization with certain agents may be an interesting approach.[21] TNF receptor targeting by TNF is an fascinating and promising approach; however, systemic administration of TNF has produced significant toxicity in the clinic.[12,13] Therefore, this approach may be limited as to how much TNF can be safely administrated.

## Sensitization of Fas-mediated cytotoxicity to cancers

Cell surface protein CD95, also known as Fas/Apo-1, is a cell surface receptor that mediates apoptotic cell death.[22] Fas ligand (FasL) is a type II integral membrane protein of the TNF superfamily [TNF, FasL, TNF-related apoptosis-inducing ligand (TRAIL)] that binds to Fas and mediates specific cytotoxicity through apoptosis and neutrophil infiltration at the tumor site.[23] Numerous investigations have been undertaken to explore the mechanism of the Fas–FasL interaction and their utilization in cancer therapy. It has been shown that IFN-$\gamma$,[24,25]

cisplatin,[25–27] camptothecin,[28] endothelin receptor antagonist (bosentan)[29] and heat-shock transcription factor 1 (HSF1)[30] can sensitize tumor cells to the cytotoxic effect of Fas–FasL. In addition, gene transfer of FasL in colon, prostate and glioma cancer cells resulted in an increase in sensitivity to Fas-mediated apoptosis *in vitro* and *in vivo*.[31–34]

Weller *et al.* demonstrated that stable transfection of Fas cDNA into glioma cell lines induced susceptibility of these cells to Fas/Apo-1 antibody-mediated apoptotic cell death.[35] Kodaira *et al.* generated a Fas–estrogen receptor-encoding vector for tamoxifen-induced cytotoxicity.[36] Cancer cells transfected with this gene express the fusion protein, bind tamoxifen at high affinity and consequently cause specific cell killing. These approaches utilize the naturally existing Fas–FasL cell death system, modifying it with receptor-targeted gene transfer. Like the TNF receptor, Fas expression is not restricted to the cancer cell surface. In addition, Fas–FasL system does not work in certain types of cancer cells as some cancer cells can escape Fas–FasL killing even though 100% of the cells are transduced. Additional investigations are required before the Fas–FasL approach can be tested in the clinic.

## Utilizing cytokine receptor to sensitize targeted cancer therapy

Targeted cancer therapy such as monoclonal antibodies and immunotoxins that target tumor-specific cell surface antigens or receptors are one of the most effective strategies against cancer.[37–40] These targeted agents require a threshold level of antigen or receptor expression on the cancer cells to achieve their antitumor activity.[41] Therefore up-regulation of antigen expression would be desirable. Several strategies have been used to up-regulate these antigens, e.g. ionizing radiation and treatment of cancer cells with IFN-$\gamma$ up-regulate antigen expression on tumor cells.[42,43] Xu *et al.* evaluated the combination of radiation therapy and immunotoxin therapy in ovarian and breast cancer cells.[42] Although only an additive effect was seen *in vitro*, *in vivo* antitumor activity and survival of tumor-bearing mice was significantly improved by the combination approach when compared with the separate therapeutic approaches. The mechanism of this synergistic effect *in vivo* was attributed to immunotoxin-induced protein synthesis inhibition and irradiation induced DNA damage resulting in depletion or down-regulation of repair enzymes, delaying recovery from the immunotoxin effect.

In the case of the IL-13R system, these receptors are constitutively overexpressed on a variety of human solid cancer cells including renal cell carcinoma,[44,45] glioma,[46–48] AIDS-associated Kaposi's sarcoma,[49] head and neck cancer,[50–52] ovarian cancer,[53] and prostate cancer.[54] To target these receptors, we have produced a recombinant fusion IL-13 cytotoxin termed IL13-PE38QQR, IL13-PE38 or IL13-PE, which is composed of IL-13 and a mutated form of *Pseudomonas* exotoxin.[52,55,56] IL-13 cytotoxin has a potent antitumor activity in IL-13R-expressing tumor cells *in vitro* and *in vivo*.[48–51,54–56] However, cytotoxicity of this molecule is limited in cancer cells that do not express or express low levels of IL-13R. We later discovered that low IL-13R-expressing cancer cells lacked the expression of IL-13R$\alpha$2 chain,[57,58] and this cytokine receptor chain plays a role in high-affinity IL-13 binding and internalization.[59,60] IL-13R$\alpha$2 chain is also found to play a key role in tumorigenicity *in vivo*.[61] Since IL-13R$\alpha$2 chain is internalized after binding to IL-13, we hypothesized that if cancer cells acquire IL-13R$\alpha$2 chain artificially, cytotoxicity of IL-13 cytotoxin to these cancer cells would be increased (Figure 1). To address this issue, we transiently or stably transfected various cancer cells with cDNA for IL-13R$\alpha$2.[50,61–63] As shown in Table 1, transfected cells acquired highly increased binding to IL-13 compared with vector only transfected control cells. Consequently, the cytotoxic activity of IL13-PE to IL-13R$\alpha$2 chain-transfected cancer cells increased by 6- to 1000-fold compared to control cells as assessed by protein synthesis inhibition and clonogenic assays (Table 1).[50,61–63] We then examined the antitumor activity of IL13-PE in immunodeficient mice harboring s.c. IL-13R$\alpha$2 chain-expressing human tumors derived from prostate (DU145) and head and neck (A253 and YCUT891) cancer cells. Consistent with *in vitro* observations, IL-13R$\alpha$2 chain-transfected tumors exhibited dramatically enhanced sensitivity to the antitumor effect of IL-13 cytotoxin by both i.p. and intratumoral routes of the drug administration.[50,63] These results demonstrated that gene transfer of IL-13R$\alpha$2 chain in cancer cells followed by targeted cytotoxin therapy represents a new useful approach for cancer therapy.

We have also tested this approach in another cytokine receptor system. IL-4 binds to IL-4R$\alpha$ chain and forms a productive complex with IL-2R$\gamma$ chain in immune cells and IL-13R$\alpha$1 chain in non-hematopoietic cells such as cancer cells.[40,64–68] We have found that IL-4R$\alpha$ chain can itself internalize after binding to IL-4.[69] IL-4R$\alpha$ chain does not require either IL-2R$\gamma$ chain or IL-13R$\alpha$1 chain for

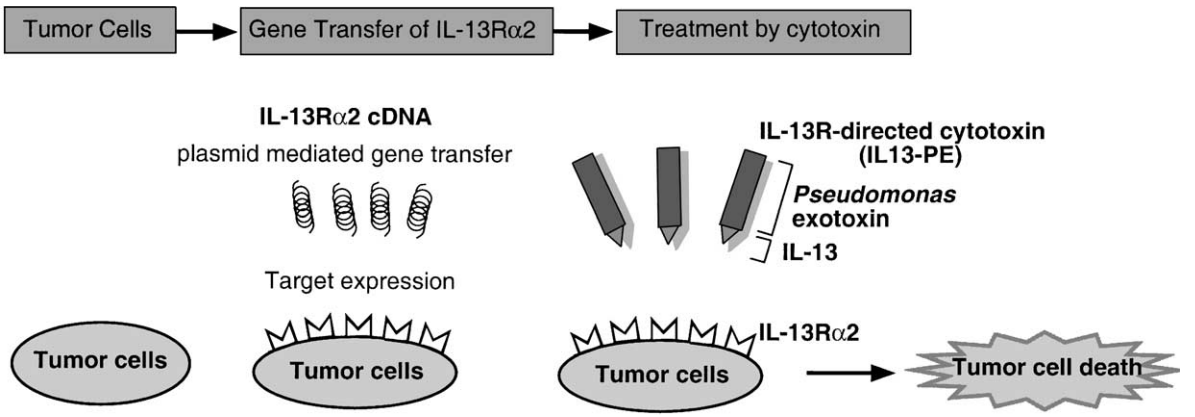


**Figure 1.** Model for cytokine receptor gene transfer and targeted cancer therapy. In this model, IL-13Rα2 gene transfected cancer cells are implanted as tumors in animal models and the mice are treated with IL-13 cytotoxin.

**Table 1.** IL-13-binding sites on cancer cells and cytotoxicity of IL-13 cytotoxin after gene transfer of IL-13Rα2 chain

| Cell type | IL-13R binding sites/cell | | $IC_{50}$ (ng/ml)[a] | |
|---|---|---|---|---|
| | Control | IL-13Rα2 transfectants | Control | IL-13Rα2 transfectants |
| Caki-1 (renal cell carcinoma)[b] | 140 | 5300 | 580 | 95 |
| T98G (glioblastoma)[b] | undetectable | 6000 | >1000 | 0.7 |
| DU145 (prostate cancer) | 30 | 16000 | >1000 | 4.0 |
| LNCaP (prostate cancer)[b] | ND[c] | ND | 200 | 3.0 |
| A253 (head and neck cancer) | 190 | 13000 | 200 | 0.2 |
| YCUT891 (head and neck cancer) | 20 | 17000 | 520 | <0.1 |
| KCCT871 (head and neck cancer) | 230 | 11000 | 300 | 0.3 |
| KB (head and neck cancer)[b] | ND | ND | 150 | 15 |
| PANC-1 (pancreatic cancer) | 160 | 38000 | 60 | <0.1 |
| CFPAC-1 (pancreatic cancer)[d] | ND | ND | >1000 | 170 |
| SU.86.86 (pancreatic cancer)[d] | ND | ND | >1000 | 90 |
| MDA-MB-231 (breast cancer) | 90 | 40000 | >1000 | 0.25 |
| MCF-7 (breast cancer)[d] | ND | ND | 130 | 8.5 |

[a] $IC_{50}$ represents a concentration of IL13-PE at which 50% inhibition of protein synthesis is observed compared to untreated cells.
[b] Data obtained from transient transfection, otherwise results from stable clones.[50,52,61–63]
[c] Not done.
[d] K Kawakami *et al.*, unpublished results.

internalization. Therefore, we hypothesized that gene transfer of IL-4Rα chain in cancer cells expressing no or low levels of this chain will sensitize these cells to the cytotoxic effect of IL-4 cytotoxin. We have previously produced and extensively characterized a IL-4 cytotoxin, IL4(38–37)-PE38KDEL which is composed of circular permuted IL-4 and a mutated form of *Pseudomonas* exotoxin. This cytotoxin is highly active in killing IL-4R-expressing tumor cells *in vitro* and *in vivo*.[40,70–77] When we tested this molecule in IL-4Rα-transfected cells, a dramatic increase in sensitivity to IL-4 cytotoxin was observed, similar to that seen in IL-13Rα2 chain transfected cells to IL-13 cytotoxin.[69]

Based on these studies, it is likely that this approach will be effective in targeting localized cancers using cytokine receptor chain gene transfer followed by targeted cytotoxin therapy.

## Conclusions

In this article, we summarized up-to-date status of unique approaches in which cytokine receptor chains are targeted for immunotherapy, gene therapy and targeted cytotoxin therapy of cancer. Although antigen-specific targeted cancer therapy has shown potent direct antitumor activity, the expression level of antigens or receptors on the tumor cell surface is often insufficient or the expression of these is heterogeneous, which limits the desirable effect of the targeted agents. Therefore, we have successfully tested a new approach in which a cytokine receptor gene transfer in tumor cells is followed by receptor-directed cytotoxin therapy *in vivo*. This approach may provide a new way of selective tumor killing without unnecessary and unwanted side effects in

normal organs. Additional studies are currently ongoing in our laboratory to apply this approach in animal models to simulate clinical situations.

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