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BIOLOGY CONTRIBUTION

COMBINED EFFECTS OF RADIATION AND INTERLEUKIN-13 RECEPTOR-TARGETED CYTOTOXIN ON GLIOBLASTOMA CELL LINES

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Purpose: Interleukin-13 receptor-targeted cytotoxin (IL13-PE38) is highly cytotoxic to human glioblastoma (GBM) cells. Although this molecule is being tested in a multicenter Phase III clinical trial (PRECISE Study) in patients with recurrent disease, the activity of IL13-PE38 when combined with radiation therapy has not been investigated.

Methods and Materials: Cytotoxicity of IL13-PE38 to GBM cell lines was assessed by protein synthesis inhibition and clonogenic assays, and the growth of GBM cells receiving radiation was assessed by thymidine uptake assays. Expression of IL-13 receptor α2 (IL-13Rα2) messenger ribonucleic acid (mRNA) in GBM cells exposed to radiation was assessed by quantitative reverse transcriptase/polymerase chain reaction (RT-PCR) and IL-13R density by radiolabeled IL-13 binding assays.

Results: Prior irradiation of GBM cell lines followed by IL13-PE38 treatment did not enhance cytotoxicity; however, concomitant 5 Gy irradiation and IL13-PE38 treatment was highly cytotoxic to T98G, M059K, A172, and LN-229 cell lines as determined by cell viability assays. There was a statistically significant decrease in number of viable cells in IL13-PE38 and irradiated cells compared with irradiated cells alone ($p < 0.05$) or IL13-PE38 treated cells alone ($p < 0.05$). In contrast, U251, SN19, and U87MG cell lines did not show any combined effect. These results were confirmed by clonogenic assays. Although three GBM cell lines—U251, SN19, and A172—showed 2.8- to 13.9-fold upregulation of IL-13Rα2 mRNA expression at 6–24 h after exposure to 5 Gy radiation, specific binding of radiolabeled IL-13 to these cell lines did not improve.

Conclusions: Our results suggest that concomitant radiation therapy and IL13-PE38 treatment may be beneficial for the treatment of patients with GBM. This strategy may be worth exploring in animal models of human glioma.

Glioblastoma, Interleukin-13 receptor, Cytotoxin, Radiation.

INTRODUCTION

Targeting cell surface proteins or antigens to cancer cells with cytotoxins or immunotoxins is being explored for brain tumor therapy (1–3). Receptors for interleukin-13 (IL-13R) have been identified in a majority of glioblastoma multiforme (GBM) cell lines and samples derived from patients undergoing surgical resection (4–7). IL-13Rα2 chain is a primary IL-13 binding and internalization component currently with no known signaling activities, and it is expressed in ~80% of GBM tumor specimens (4, 8–12). In contrast, normal astrocytes and brain tissues do not seem to express this receptor chain (6, 7). To target IL-13R, we have developed chimeric proteins termed IL13-PE38, in which human IL-13 and a mutated form of *Pseudomonas* exotoxin are conjugated (13, 14). IL13-PE38 has shown potent antitumor activity against IL-13R-expressing tumors *in vitro* and *in vivo* (13–17). Four Phase I/II clinical trials using IL13-PE38QQR (a modified form of IL13-PE38 in which c-terminus of PE is mutated without any change in cytotoxicity) in patients with recurrent GBM were recently completed which show that convection-enhanced delivery (CED) of IL13-

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PE38QQR can be administered safely in brain tumors and in normal brain surrounding the tumor cavity (18–20). Patients have tolerated this therapy well and prolonged survival has been observed in patients receiving IL13-PE38QQR (18–20). Currently, a Phase III randomized evaluation of CED of IL13-PE38QQR with survival endpoint study (PRECISE) is ongoing at numerous centers in the United States, Canada, and Europe to test the efficacy of IL13-PE38QQR in a large patient population with recurrent GBM.

Radiation therapy is one of the main treatment modalities for primary GBM (21, 22). It has been demonstrated that the expression levels of several tumor-surface proteins, which can be targeted with cytotoxins or immunotoxins are increased after irradiation of tumor cells. For example, radiation upregulates expression levels of receptors for epidermal growth factor (23), transferrin, insulin-like growth factor 1, and interleukin-4 on GBM cells (24). Therefore, the utilization of radiation in GBM therapy is not limited to its therapeutic modality; it may also act as a sensitizer for cytotoxin or immunotoxin therapy of GBM. Although the effect of IL13-PE38 on GBM is being vigorously pursued, its utility in combination with radiation therapy has never been investigated.

In this study, we investigated the effect of radiation on IL13-PE38-induced cytotoxicity in GBM cells *in vitro*. The effect of IL-13 and IL13-PE38 on the radiation-mediated growth inhibition of GBM cells was then investigated. Finally, the concomitant effect of radiation and IL13-PE38 in GBM cells was assessed. Additionally, IL-13Rα2 messenger ribonucleic acid (mRNA) expression levels and IL-13R expression in GBM cell lines were evaluated. These studies are critically important for further development of combined approach of radiation and IL13-PE38 therapy in patients with GBM.

METHODS AND MATERIALS

Cell, reagents, and radiation

Human glioblastoma cell lines (U251, A172, T98G, U87MG, U373, SN19, LN-229, and M059K) were maintained in Eagle's minimal essential medium (T98G and U373), RPMI-1640 (U251, A172, U373, and SN19), Dulbecco's modified Eagle's medium (LN-229), or Dulbecco's modified Eagle's medium:Ham's F-12 (1:1 volume) (M059K) medium supplemented with 10% fetal bovine serum, L-glutamine, and antibiotics. Recombinant IL13-PE38 was produced and purified in our laboratory (13, 14). Recombinant IL-13 was purchased from Peprotech, Inc. (Rocky Hill, NJ). For irradiation, cells were exposed at room temperature to the Gammacell 1000 Elite Model II ^{137}Cs irradiator (Nordion International, Inc., Ontario, Canada).

Protein synthesis inhibition assays

The cytotoxic activity of IL13-PE38 was tested by the inhibition of protein synthesis as previously described (25). Typically, 10^4 cells were cultured in leucine-free medium with or without various concentrations of IL13-PE38 for 22 h at 37°C. Then 1 μCi of [^{3}H]leucine (NEN Research Products, Boston, MA) was added to each well and incubated for an additional 4 h. Detached cells were harvested and radioactivity (cpm) incorporated into cells was measured by a β plate counter (Wallac, Gaithersburg, MD). The percent cpm of treated cells was calculated by comparing cpm of untreated control cells, which were considered 100% (13, 15).

^{3}[H]thymidine uptake assays

Glioblastoma multiforme cells (10^4 cells/well) were cultured for 48 h before [^{3}H]thymidine was added to each well. Cells were cultured for an additional 24 h. Plates were harvested using a Skatron cell harvester (Skatron, Inc., Sterling, VA) to filter mats, and radioactivity incorporated into cells was measured by a β plate counter.

Clonogenic assays

The *in vitro* cytotoxic activity of IL13-PE38 against U251 and A172 GBM cell lines receiving radiation was also determined by a colony-forming assay (26). Five hundred cells were plated in triplicate in 100-cm^2 cell culture dishes with 7 mL of RPMI-1640 containing 10% fetal bovine serum and were allowed to attach for 12–16 h. Cells exposed to IL13-PE38 (0–10 ng/mL) for 8–12 days at 37°C in a humidified incubator were then washed and stained with 0.25% crystal violet. Colonies consisting of $>$50 cells were scored. Both cells lines generated a slightly different and reproducible number of colonies. U251 cells formed 116 $\pm$ 13 colonies, whereas A172 cells made 72 $\pm$ 8 colonies.

Quantification of mRNA expression

IL-13Rα2 mRNA expression levels in GBM cells were determined by quantitative reverse transcriptase/polymerase chain reaction (RT-PCR) using a TaqMan™ probe (5′-FAM TGAGTGGAGTGATAAACAATGCTGGGAAGG TAMARA-3′) and primer pairs (5′-TGCTCAGATGACGGAATTTGG-3′, and 5′-TGGTAGCCAGAAACGTAGCAAAG-3′) complementary to IL-13Rα2 (27). Total ribonucleic acid (RNA) was reverse transcribed into the complementary deoxyribonucleic acid (cDNA) primed with random hexamer (Applied Biosystems, Foster City, CA). Quantitative PCR reactions were performed using the ABI PRISM 7700 sequence detection system (Applied Biosystems). Twenty-five ng of total cDNA was added to a reaction volume (25 μL) containing 1$\times$ Taqman PCR master mix, 300 nM primer pairs, and 100 nM probe. To calculate the copy number of the IL-13Rα2 gene, a standard curve was generated using a plasmid containing whole IL-13Rα2 cDNA (10). To normalize the cDNA concentration in each sample, the copy number of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was quantified.

Radioreceptor binding assays

Recombinant human IL-13 was labeled with ^{125}I (Amersham Pharmacia Biotech, Arlington Heights, IL) using IODO-GEN reagent (Pierce Chemical Co., Rockford, IL) as previously described (10). The specific activity of the radiolabeled IL-13 was estimated to be 20.5 μCi/μg of protein. For binding experiments, 5 $\times$ 10^5 cells in 100 μL binding buffer (RPMI-1640 containing 0.2% human serum albumin and 10 mM HEPES) were incubated with 200 pM ^{125}I-IL-13 with or without unlabeled IL-13 (40 nM) at 4°C for 2 h. Cell-bound ^{125}I-IL-13 was separated from unbound by centrifugation through a phthalate oil gradient, and radioactivity was determined with a gamma counter (Wallac, Gaithersburg, MD).