ANTICANCER RESEARCH *41*: 3471-3480 (2021)
doi:10.21873/anticanres.15134

IL13 Fused *Pseudomonas* Exotoxin Targets Various Cancers *In Vitro*

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Abstract. *Background/Aim: Pseudomonas exotoxin (PE) is one of the most widely used toxins in the construction of therapeutic fusion proteins in pre-clinical studies followed by phase trials. In principle, PE acts by blocking protein synthesis through catalyzing the inactivation of elongation factor-2 (EF-2). The interleukin-13 fused PE (IL13-PE) cytotoxin was previously designed to target GBM cells. In this study, the cytotoxic effects of IL13-PE were evaluated in 5 different types of cancers and the therapeutic effects were further analyzed in a lung cancer cell line, NCI-H460. Conceptually, in another lung cancer cell line (A549), IL13Rα2 was overexpressed by lentiviruses (A549-IL13Rα2) and evaluated for cytotoxic efficacy of IL13-PE. Materials and Methods: The expression profile of IL13Rα2 in different cancer cell lines was determined by RT-PCR. Secretable toxin fusion was expressed in the toxin resistant HEK-293T cell line (293T-TxR) by using a plasmid coding for IL13-PE and IRES-GFP (LV-IL13-PE-IRES/GFP). Next, the cells were shown to produce and secrete functional IL13-PE by dot blot analysis, followed by cell viability assays and cell death analysis. Results: Upon treatment with IL13-PE, a significant decrease in cell viability was selectively demonstrated in cancer cells with cognate receptor expression. IL13-PE treatment increased the apoptotic/necrotic cell populations in the NCI-H460 cell line. Conclusion: Our results demonstrate that IL13-PE can be a therapeutic target for tumors bearing mostly IL13Rα2 positive cell populations. Our findings also suggest a cell-based delivery option for the recombinant toxins in the treatment of different cancers which can provide a solution for the clinical use of toxin therapy.*

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Key Words: Pseudomonas exotoxin, toxin, recombinant toxin, IL13, IL13Rα2, cancer.

Traditional cancer treatments often result in severe side effects and high tumor recurrence rates due to the off target approaches. Extensive efforts have been made to develop targeted therapies while reducing unwanted ramifications (1-4). One of the most well-known cancer-targeting strategies both in preclinical and clinical development is the use of targeted cytotoxins. Cytotoxins have the ability to target receptors and/or antigens that are specifically expressed in cancer cells (5, 6). *Pseudomonas* Exotoxin (PE), originally produced by the bacteria *Pseudomonas Aeruginosa*, is among the most commonly studied toxins (7-10). PE catalyzes the inactivation of elongation factor-2 (EF-2) and causes cell death by blocking protein synthesis. PE can be directed to the receptors that are specifically expressed in tumors (11, 12). This strategy provides strong cytotoxic activity, which is ensured to only act on malignant cancer cells.

One of the receptors targeted in several cancers is IL13Rα2, a variant of the IL-13 receptor. Although the overexpression of IL13Rα2 has been primarily reported in glioblastoma, breast, ovarian, colorectal and pancreatic cancer, more studies are needed for a better understanding of the clinical importance of IL13Rα2 targeting in a variety of cancers (13-15). As shown in another study, more than 50% of glioblastoma (GBM) cells express IL13Rα2. Also, deficiency of IL-13Ra2 in normal brain cells enables IL13-PE to selectively target and kill GBM cells without damaging healthy tissues. In addition, IL13-PE treatment has resulted in a significant increase in mouse survival in clinically relevant GBM tumor model (16). However, in the clinic, PE-based cytotoxins have been used successfully to treat a variety of malignancies, including prostate, ovarian, leukemia, and Hodgkin lymphoma (17-20)(NCT00003020, NCT00104091, NCT00924170, NCT01051934, NCT02858895, NCT01061645). Preclinical

testing has shown that IL13-PE is highly toxic to cells expressing IL-13Rα2 in culture and *in vivo*. Early phase clinical trials show that IL13-PE is well tolerated despite some side effects (21-23). In this study, the targetability of IL13Rα2 in other cancers was firstly evaluated and IL13-PE fusion toxin was directed to cancer cells homing IL13Rα2. Following the treatment with IL13-PE, effective cell death was observed in cancer cells in correlation with receptor presence.

In this study, the expression profiles of IL13Rα2 in cells of various cancer types were monitored. We report that cancer cells can be targeted with IL13-PE through IL13Rα2 in a broad range of cancer types. Significant reduction in cell viability was detected in receptor-expressing cancer cells following IL13-PE treatment. Accordingly, we showed a reasonable fact of using the IL13-PE toxin fusion in targeted therapy strategies against different cancers bearing the cognate receptor, IL13Rα2. To our knowledge, although it has been previously shown that IL13Rα2 can be targeted in lung cancer, and one study suggest IL13Rα2 specific monoclonal antibody therapy, no detailed study of IL13Rα2 targeted recombinant toxins have been reported against lung cancer (15, 24). The anti-cancer effects of IL13-PE were then analyzed in lung cancer cell lines. Further studies can be followed by *in vivo* tumor modeling of cancer cells expressing the target receptor. These results can potentiate the cell-based delivery of IL13-PE targeted toxin in a continuous and onsite delivery method and serve as a basis for clinical studies.

Materials and Methods

Cell culture. Glioblastoma (U87, LN229), pancreas (ASPC-1, PANC-1), lung (NCI-H460, A549), breast (MDA-MB-157 and 231) and melanoma (Sk-Mel-2, MeWo) cancer cell lines were obtained from the American Type Culture Collection (ATCC). Growth media for each cell line were used as recommended by the manufacturer. U87, LN229, A549, Sk-Mel-2 and MeWo cells were grown in Eagle's Minimum Essential Medium (EMEM); AspC-1 and NCI-H460 in RPMI-1640 medium; Panc-1 cell in Dulbecco's Modified Eagle's Medium (DMEM) and finally MDA-MB-157, MDA-MB-231 cells in Leibovitz's L-15 Medium (Gibco, New York, NY, USA). In addition, all cells were cultured in 37˚C at 5% CO_2 in growth media containing 10% fetal bovine serum (FBS; Gibco) and 1% Penicillin/Streptomycin (PS; Gibco).

RNA extraction and reverse transcription-PCR analysis. When the cells reached 80-90% confluency, they were passaged and expanded. cDNA samples for RT-PCR were prepared from the expanded cells. Firstly, total RNA was used as recommended by the manufacturer using the RNeasy RNA extraction kit (Qiagen, Venlo, the Netherlands). Total RNA was reverse transcribed to obtain cdNA using the High-Capacity cDNA reverse transcription kit (Thermo Scientific, Waltham, MA, USA). After the cDNA library was obtained, PCR was applied to determine the IL13Rα2 receptor expression levels in cells using the following primer sets; sense: 5'-ATGGCTTTCGTTTGCTTGGCT-3', antisense: 5'-TGATCCAAGCCCTCATACCAG-3' primer pairs were used (534 bp). The GAPDH primer pair (sense: 5'GTCAGTGGT GGACCTGACCT-3', antisense: 5'-TGCTGTAGCCAAATTCGTTG-3'), produces a 245 bp fragment and was used as a positive control.

Transfection: Collection of IL13-PE conditioned medium from toxin resistant 293T-TxR cells. To obtain toxin fusion from 293T-TxR cells, these cells were seeded in 150 mm petri dishes to be transfected with LV-IL13-PE. 24 h after seeding, LV-IL13-PE plasmid DNA was transferred to cells by the $CaCl_2$-based method and transfection success was monitored with GFP expression. In addition, as a control, 293T-TxR cells were transfected with a plasmid coding GFP. Serum-free conditioned medium from 293T-TxR cells engineered to express secretable forms of IL13-PE was collected 24 h after transfection and concentrated with centrifugal filter units (Amicon, Millipore, Sigma, Darmstadt, Germany). GFP conditioned media was also collected and used properly for each control run.

Dot blot analysis: IL13-PE protein concentration. Mediums obtained and concentrated from 293T-TxR cells were used to determine the secretion of IL13-PE by the cells. For this, concentrated conditioning medium was loaded onto a PVDF membrane (0.5 and 3 μl) and immunoblotted using antibody against PE (P2318, Sigma-Aldrich, St. Louis, MO, USA 1:10,000 antibody dilution). The antibody incubation was performed at room temperature for 1 h on a shaker. Following the primary antibody incubation, the membrane was further incubated with HRP-conjugated goat anti-rabbit secondary antibodies (Ab6702, Abcam, 1:3,000 antibody dilution) for 30 min at room temperature. Band densities were measured using ImageJ version 1.52q. Protein concentration was then determined using a standard curve of PE toxin (Sigma).

Lentiviral packaging. Lentiviral packaging was carried out as previously described (16). Briefly, to obtain the LV-IL13Rα2-iGFP virus, 293T cells were seeded in 150 mm petri dishes. After 24 h, the medium was refreshed. Packaging and expression plasmids were transfected (CMV-Delta with VSVG and IL13Rα2 plasmid) using $CaCl_2$. After 16 h, the medium was replaced with a serum free medium; it was then collected and passed through a 0.45 μM filter. Finally, the medium containing the virus was centrifuged at 28,000 × *g* for 1 h by ultracentrifuge concentrated.

Transduction and IL13Rα2 overexpression. After viral tittering on 293T cells, A549-wt cells were infected with the virus at an MOI of 2, in the presence of 4 mg/ml protamine sulphate (Sigma). The next day, cells were washed with PBS (GIBCO) and the medium was replaced with new growth medium. After 18-24 h, GFP expression was visualized under fluorescence microscopy. To validate the IL13Rα2 transfer, cDNA samples were prepared according to the protocol given above and RT-PCR of the receptor transcripts was performed.

Cell viability assays. To determine toxin resistance of 293T-TxR cells (16) were treated with a range of PE toxin (Sigma) (0, 50, 100, 500 and 1,000 ng/ml) prior to LV-IL13-PE transfection. As for control, wild type HEK-293T cells were used. All cells were seeded at a density of 1×10^4 into 96-well plates. Twenty-four hours later, they were treated with the given dose of PE for 48 h. Subsequently, cell viability was measured using an ATP-coupled luminescent reagent (cellTiter-Glo; Promega, Madison, WI, USA) and analyzed with Spectromax. To analyze cell death on cancer cells treated with

IL13-PE, all cancer cells were seeded in 96-well plates at a density of 1×10^4. After 24 h, the media of the cells was removed and they were treated with different concentrations (10, 25 and 50 ng/ml) of IL13-PE or GFP (control) conditioned medium. Cell viability was then measured as described above.

Flow cytometry. NCI-H460 cells were seeded in 6-well cell culture dishes at 2×10^5 cells / well and incubated at 37˚C in 5% CO_2 for 24 h. The culture medium was then discarded, and the cells were treated with 25 ng/ml IL13-PE and commercial purified toxin (Diphteria Toxin, DT; Sigma). Forty-eight hours upon treatment, Annexin V-FITC/Propidium Iodide (PI) early apoptosis double staining was applied following the manufacturer's instructions (Annexin V-FITC Early Apoptosis Detection Kit, Cell Signaling Technology, Danvers, MA, USA). Percentages of early apoptotic, late apoptotic/necrotic and live cells were determined by the FlowJo analysis software (Tree Star, FlowJo LLC, Ashland, OR, USA).

Western blotting. NCI-H460 cells were seeded at 2×10^5 cells/well in 6-well plates and incubated at 37˚C in 5% CO_2 for 24 h. The culture medium was then discarded and cells were treated with 25 ng/ml IL13-PE conditioned medium. After 48 h, protein lysates were obtained in RIPA lysis buffer (Thermo Fischer Scientific #89900) and 30 μg of each protein sample was loaded and run on SDS-PAGE. The Bio-Rad semi-dry western blotting protocol was then applied. Membranes were blocked with 5% BSA and incubated in antibody solutions. As of primary antibodies; cPARP (CST #9542) (1:2000) and β-actin (13E5) (CST #4970) (1:2000) were used. For secondary antibodies; anti-rabbit IgG, HRP-linked antibody (CST #7074) (1:2000), and anti-mouse IgG, HRP-linked antibody (GenDEPOT #W3903) (1:2,000) were used. After applying the ECL substrate (Bio-Rad, Hercules, CA, USA), the protein bands were detected using the ChemiDoc MP Imaging System (Bio-Rad). Comparative band intensities were quantified by ImageJ.

Statistics. Significance was determined by the Student *t*-test when comparing two groups and One way ANOVA for more than 2 group comparisons. Data were expressed as mean±SEM. Differences were considered significant when $*p<0.05$, $**p<0.01$ and $***p<0.001$.

Results

IL13Rα2 is a potential target for cancer cells from multiple tumor origins. A panel of cancer cells was used to investigate the therapeutic potential of IL13-PE therapy against various types of cancers. For this aim, 5 different cancer types from GBM (U87, LN229), pancreas (ASPC-1, PANC-1), lung (NCI-H460, breast (MDA-MD-157 and 231) and melanoma (Sk-Mel-2, MeWo) were examined for the presence of IL13Rα2 expression. The U87 glioblastoma cancer cell line was used as a positive control for receptor expression and LN229 was used as a negative control as previously shown (10). Expression of IL13Rα2 was detected in pancreatic, lung, breast and melanoma cancer cell lines (Figure 1A, B). Therefore, a range of cancer cell lines that could be targeted with IL13-PE were identified.

Toxin resistant cells can secrete functional IL13Rα2 targeted PE (IL13-PE). Endogenous production of PE toxin inactivates EF-2 and blocks protein synthesis, which then results in subsequent cell death. For this reason, even endogenic levels of IL13-PE can result in cell death and this requires toxin resistance for cell-based delivery of IL13-PE. Establishment of toxin resistant cells can be achieved by point mutation of endogenous EF-2. In a previous study, single-stranded oligonucleotides (ssODN) coding mutant EF-2 (ssODN-mEF-2) were used to transform normal HEK-293T cells into a toxin-resistant variant (these cells are herein referred to as 293T-TxR) (10). In this study, we examined the toxin resistance of the cells compared to wild type HEK-293T prior to IL13-PE condition medium collection from 293T-TxR. After 48 h of treatment with PE toxin (0-1,000 ng/ml), the viability of 293T and 293T-TxR cells decreased. The most significant reduction in cell death ratio (10%) was detected in 1,000 ng/ml toxin treated 293T cells (Figure 2A). In addition, 293T-TxR cells could survive 97% at the same doses (Figure 2A) ($*p<0.05$, $**p<0.01$ and $***p<0.001$). These results showed that 293T-TxR cells are highly resistant to IL13-PE and can be further engineered to express functional PE cytotoxins.

Successful LV-IL13-PE transfection was followed by GFP expression. At the same time, 293T-TxR cells were also transfected with LV-GFP as control (Figure 2B). 24 h after LV-IL13-PE transfection of 293T-TxR cells, the conditioned medium was collected and concentrated. To detect and quantify IL13-PE secretion from 293T-TxR cells, PE presence was examined in conditioned medium by dot blot analysis. Conditioned medium from LV-GFP transfected 293T-TxR cells was used as negative control (Figure 2C). This data showed that 293T-TxR cells can endogenously produce and release the IL13-PE toxin fusion.

IL13-PE has anti-cancer effects on various cancer cell lines. Cancer cells expressing target receptor were investigated for the therapeutic effects of IL13-PE. We found that cancer cells treated with IL13-PE at different concentrations (10, 25, 50 ng/ml) caused a significant decrease in cell survival (Figure 3). In particular, a significant reduction of 47% and 42% in cell viability of lung cancer cells (NCI-H460) and melanoma cancer cells (Sk-Mel-2), respectively, was detected in 25 ng/ml IL13-PE treated groups (Figure 3). To our knowledge, this is the first report indicating the therapeutic potential of IL13-PE that can be further studied for the treatment of different cancers.

IL13-PE functions on cancer cells expressing the target receptor IL13Rα2. Our results showed that IL13-PE functions on selectively IL13Rα2 expressing cells. Among the cell lines tested, we detected NCI-H460 lung cancer cells with the most significant cell death induced by IL13-PE. Conceptually, we then engineered wild type A549 cells (A549-wt) lacking the target receptor IL13Rα2 to overexpress IL13Rα2 using

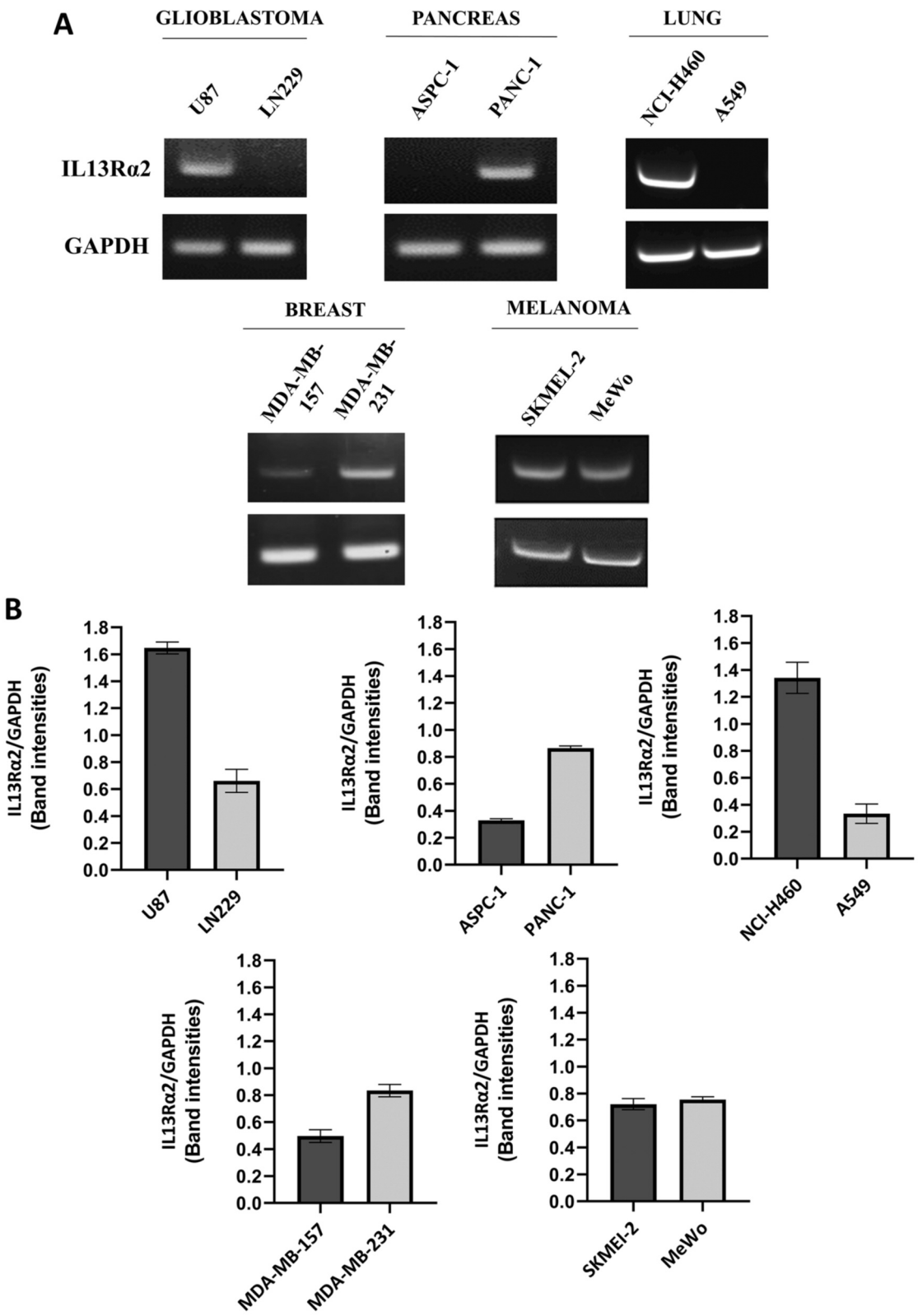

Figure 1. *IL13Rα2 transcripts in various cancer cell lines. A) RT-PCR analysis of IL13Rα2 expression from 5 different cancer lines including glioblastoma (U87, LN229), pancreas (ASPC-1, PANC-1), lung (NCI-H460), breast (MDA-MB-157, MDA-MB-231) and melanoma (Sk-Mel-2, MeWo) and B) Relative band intensities by Image J analysis. Data are expressed as mean±SEM.*

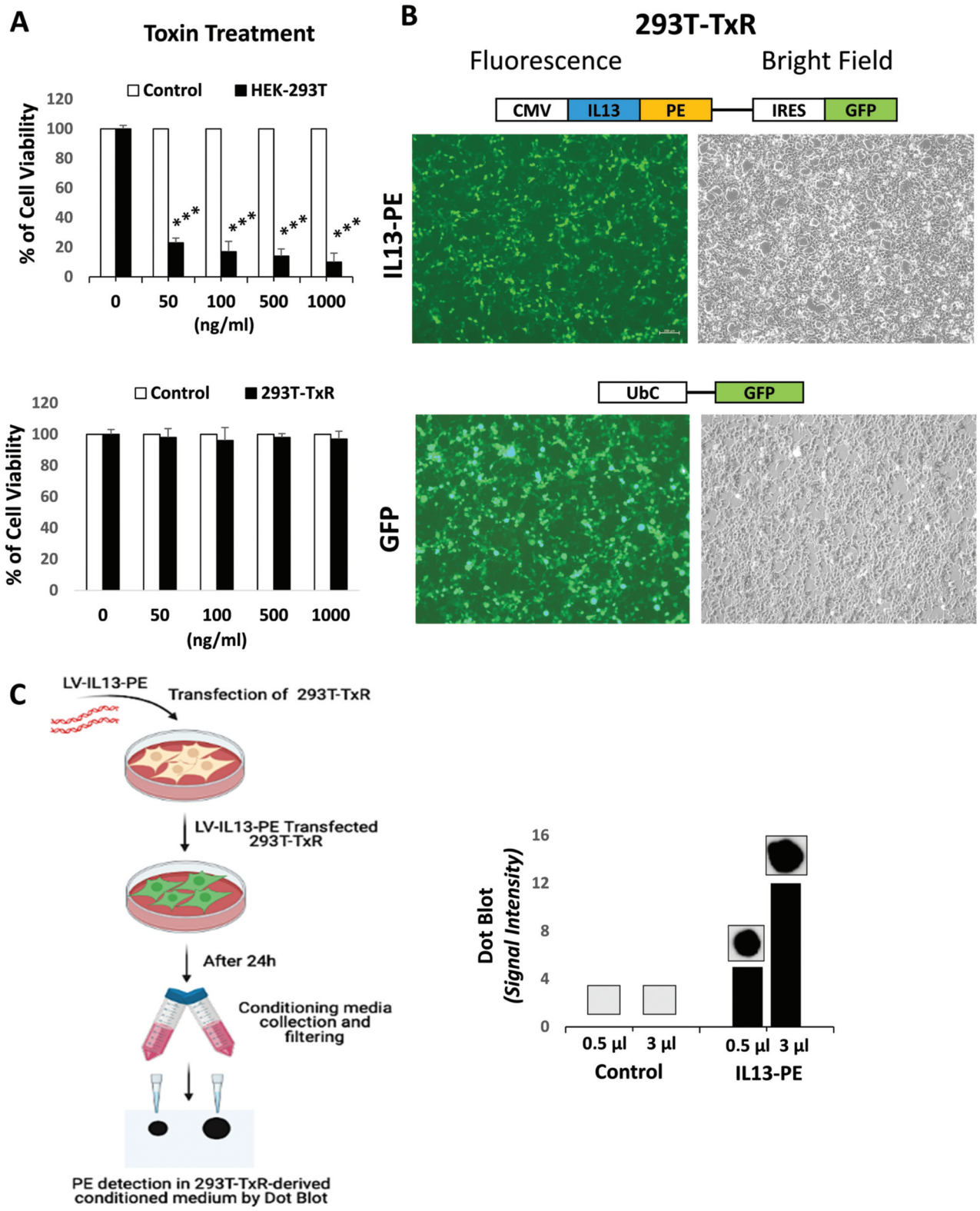

Figure 2. *Secretion of IL13-PE by toxin resistant 293T-TxR cells. A) Cell viability analysis after PE toxin treatment in HEK-293T cells and toxin resistant HEK-293T-TxR cells. B) Microscope images of 293T-TxR cells transfected with LV-IL13-PE-iGFP or LV-GFP. C) Dot blot detection of the IL13-PE secreted by HEK-293T-TxR-IL13-PE cells compared to GFP conditioned medium (negative control). Data are expressed as mean±SEM. Differences were considered significant at *p<0.05, **p<0.01 and ***p<0.001.*

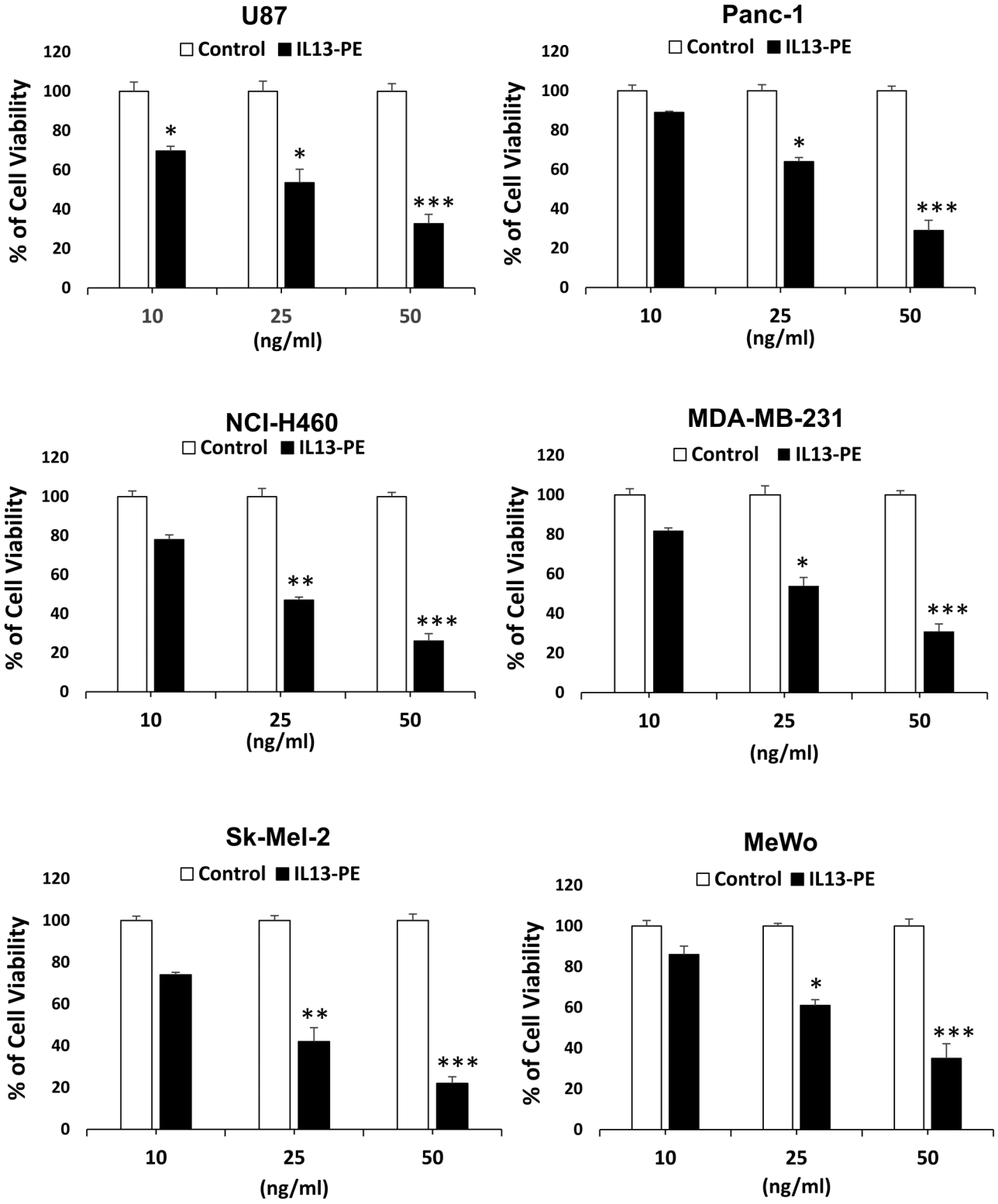

Figure 3. *Cell death analysis of IL13-PE treated various cancer cell lines expressing the target receptor. Plots indicate cell viability of the various cancer lines (U87; GMB, Panc1; Pancreas, NCI-H460; lung, MDA-MB-231; breast, Sk-Mel-2 and MeWo; melanoma) following the treatment with increasing amounts of IL13-PE or control (GFP) conditioned medium (10-50 ng/ml). Data are expressed as mean±SEM. Differences were considered as significant at *p<0.05, **p<0.01 and ***p<0.001.*

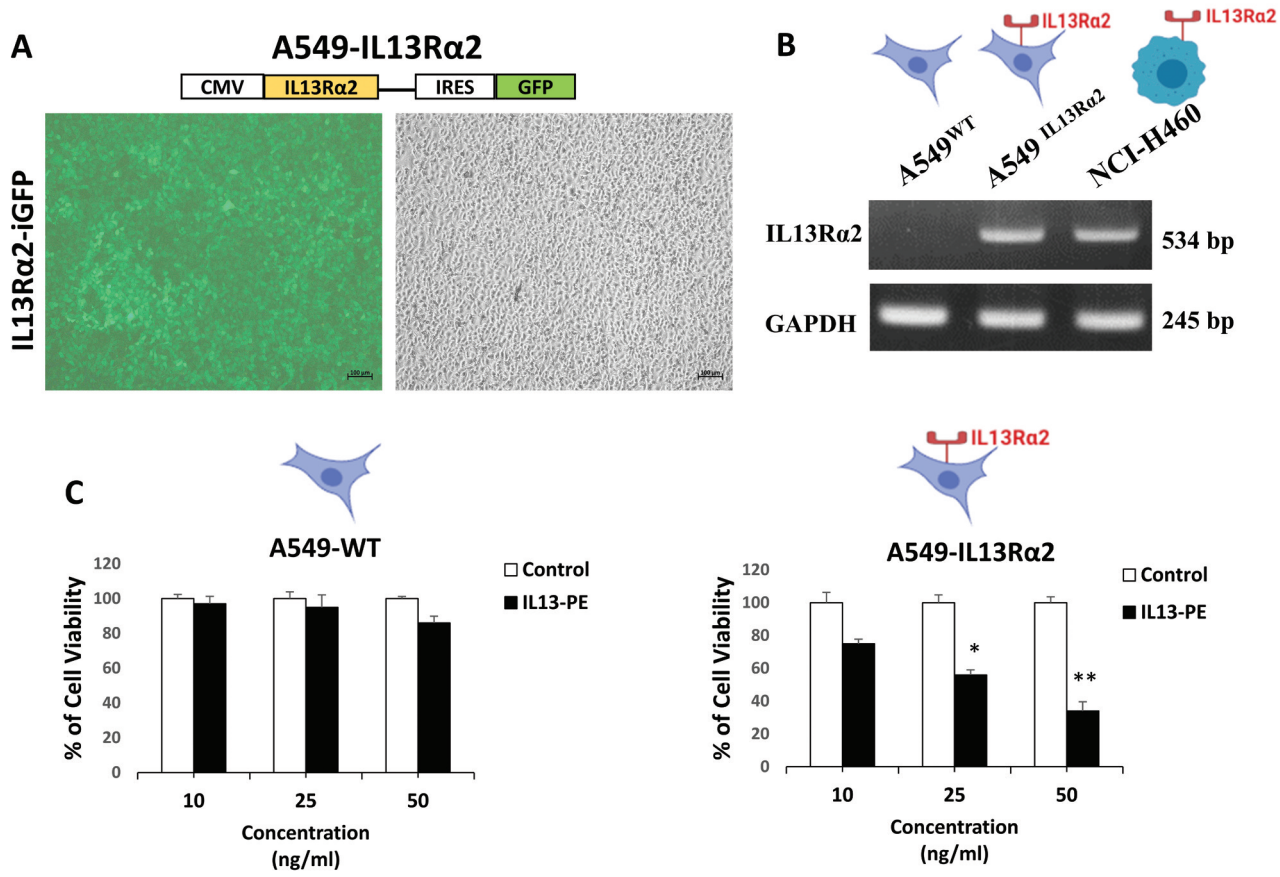

Figure 4. *IL13-PE induces cell death on the receptor overexpressed A549 lung cancer cell line (A549-IL13Rα2). A) Fluorescent and brightfield microscopic images of A549 cells infected with LV-IL13Rα2 lentivirus. B) Detection of IL13Rα2 transcript by RT-PCR for A549-wt and A549-IL13Rα2 lung cancer cell lines. For positive control, an IL13Rα2 expressing NCI-H460 lung cancer cell line was also used. C) Plots showing the cell viability of IL13-PE treated (10-50 ng/ml) A549-wt and IL13Rα2 overexpressing A549-IL13Rα2 cells. Data are expressed as mean±SEM. Differences are considered significant at *p<0.05, **p<0.01 and ***p<0.001.*

LV- IL13Rα2-iGFP lentivirus. Successful infection was followed by GFP expression under fluorescence microscopy (Figure 4A). The established A549-IL13Rα2 cell line showed the existence of IL13Rα2 expression (Figure 4B). Subsequently, A549-wt and A549-IL13Rα2 cells were treated with IL13-PE or GFP conditioned medium. As a result, A549-wt cells sustained their viability (86%) when treated with even the highest IL13-PE concentration, 50 ng/ml. Conversely, A549-IL13Rα2 cells showed significantly decrease in viability (34%) at the same concentration (Figure 4C). Taken together, these results revealed that IL13-PE is functioning in a target specific manner and can act on cancer cells expressing IL13Rα2.

IL13-PE induces apoptotic and necrotic cell death in the NCI-H460 lung cancer cell line. The lung cancer cell line NCI-H460 was used to examine cell death induced by IL13-PE. We firstly investigated the natural toxin behavior on NCI-H460 cells. Cell viability was analyzed upon treatment with different concentrations of diphtheria toxin (DT) (Figure 5A). We then determined the optimum DT dose (2 ng/ml) for cell death analysis considering the viability plot. Toxin treated cells were subjected to flow cytometry at the same time with IL13-PE treated cells. Flow cytometry analysis showed that both IL13-PE and DT treated NCI-H460 cells for 48 h have apoptotic and necrotic cell populations (Figure 5B). We also analyzed apoptosis in IL13-PE treated NCI-H460 cells by western blotting. Cleaved PARP, one of the biochemical indicators of downstream apoptosis, was detected in IL13-PE treated cells and was significantly higher when compared to control treatments (Figure 5C).

Discussion

Recombinant toxins are becoming promising tools for targeted therapy approaches against cancer. The therapeutic potential and delivery strategy of IL13 fused PE-toxin

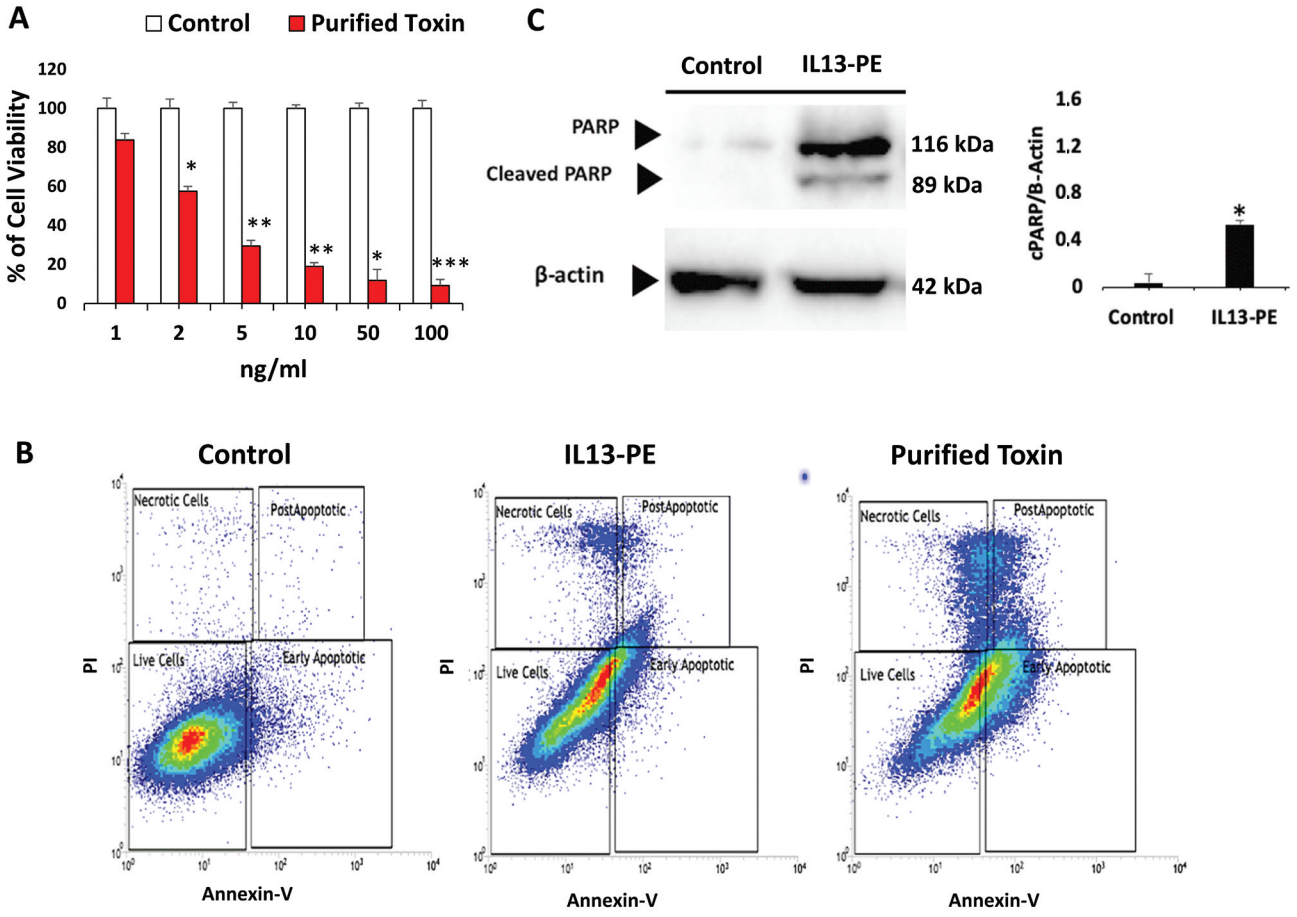

Figure 5. *Cell death analysis in IL13-PE treated NCI-H460 lung cancer cell line. A) Toxic dose of purified toxin (Diphteria toxin; DT, Sigma) in the NCI-H460 cell line. Plot showing cytotoxicity of DT in NCI-H460 cell line upon treatment with increased doses (1-100 ng/ml) of DT for 48 h. B) Flow cytometry analysis of cell death in NCI-H460 cells after 48 h of treatment with IL13-PE (25 ng/ml) and purified toxin (2 ng/ml). Annexin-V or Propedium Iodide staining indicate apoptotic and necrotic cell death, respectively. C) Detection of cleaved PARP (cPARP) in IL13-PE treated NCI-H460 cells. Plot shows quantification of cPARP levels by Image J (cPARP band intensities are normalized to beta-actin). In all IL13-PE treatment groups, GFP conditioned medium was used as a control treatment. Data are expressed as mean±SEM. Differences were considered as significant at *p<0.05, **p<0.01 and ***p<0.001.*

against glioblastoma brain tumors has already been demonstrated. In this study, the *in vitro* therapeutic effects of the IL13-PE targeting IL13Rα2 were investigated in different types of cancers. Our results showed that IL13-PE induces a significant reduction in the viability of certain cancer cells from different tumor origins. This therapeutic action depends on the expression of the target receptor, IL13Rα2. We further analyzed cell death in a lung cancer cell line (NCI-H460), where one of the greatest cell death percentages was observed. According to the results obtained, we suggest the potential use of IL13-PE against a variety of cancer cells in targeted therapy approaches. In future studies, *in vivo* tumor models and delivery systems can be applied and therapeutic effects of IL13-PE can be thoroughly investigated. Additionally, analysis of IL13Rα2 expression from whole tumor extracts can provide options for determination of possible tumor targets. This may eventually suggest a solution to switch the system to a clinical basis.

In recent decades, chemotherapy and radiotherapy have been used as the traditional cancer treatment methods. However, these applications can also affect healthy cells and cause various side effects. Consequently, targeted therapy strategies are becoming promising anti-cancer tools. In line with this, we applied a non-toxic strategy suggesting the use of IL13-PE to selectively target cells with cognate receptor expression. One of the advantages of the fusion is also its cancer selective targeting ability since normal healthy cells lack the cognate receptor. Secondly, inside the cell PE acts using a general mechanism, through EF-2 inactivation and subsequent inhibition of protein

synthesis. This means that it can function in many cells once it enters the cytosol, regardless of the highly mutagenic profile of cancer cells. Principally, IL13Rα2 expression mediates IL13-PE endocytosis and enables targeting cancer cells selectively since healthy cells lack of the receptor. Considering these moieties of IL13-PE toxin fusion, our results contribute to the determination of possible targets for IL13-PE.

Targeted toxins also have a sensitizing effect to some chemo-agents on cancer cells. This brings an advantage in therapeutic options. For instance, epidermal growth factor receptor (EGFR) targeted DT toxin fusion (EGF-DT) was shown to sensitize TRAIL (tumor necrosis factor related apoptosis inducing ligand) mediated apoptosis *via* downregulation of FLIP [FLICE (FADD-like IL-1β-converting enzyme) inhibitory protein] (25, 26). Subsequent activation of caspase 8 enhanced the death signals in cancer cells. Similarly, using IL13-PE in combination with other anti-cancer agents may provide more effective killing of cancer cells in a synergistic manner. As previously discussed, the technical advantage of modifying stem cells to secrete multiple therapeutics can be a strategy for the delivery of IL13-PE in combination with apoptosis inducers.

Heterogeneous tumor populations have become one of the biggest limitations in the currently developed targeted therapies. However, what is more important in developing targeted therapy strategies for cancer is identifying the high ratio of targetable cells within a tumor. Furthermore, targeting most of the cells in a heterogenous population may reduce the tumor burden. Therefore, analysis of IL13Rα2 receptor expressions in human tumor tissue samples and investigations into the *in vivo* therapeutic effects of IL13-PE toxin may be promising for deciding on the potential use of IL13-PE for certain type of tumors. On that account, our data and follow up pre-clinical studies may be decisive for individualized targeted therapy approaches.

Regarding systemic toxicity, molecular half-life and inadequate tissue penetration, one concern would be to optimize the effective delivery of IL13-PE for therapeutic use against human cancers. For this purpose, recombinant toxins have been capacitated by their stem cell-mediated secretion as previously shown in glioblastoma (GBM) brain tumor. Accordingly, our results potentiate the stem cell-based delivery of IL13-PE which enables on site and long-term secretion of toxin fusion. In this way, the toxin can be more effective by reaching foci and releasing cytotoxins continuously, especially in solid tumors.

Conclusion

Based on previous data demonstrating a significant therapeutic efficacy of IL13-PE fusion protein in a clinically-relevant GBM tumor resection model, we investigated the possible efficacy of IL13-PE on different cancers, *in vitro*. IL13-PE was shown to act therapeutically on certain cancer cell lines *via* targeting IL13Rα2 in a cancer-selective manner. Therefore, IL13-PE could be a promising therapeutic candidate. Our findings also support further studies of IL13-PE to determine its potential use in the clinical setting, especially for solid tumors.

Conflicts of Interest

The Authors declare no potential conflicts of interest.

Authors' Contributions

D.U.: Collection and assembly of data, Data analysis and interpretation, Manuscript writing, Revision of the manuscript, Final approval of the manuscript. N.K.: Conception and design, Financial support, Provision of study material, Assembly of data, Data analysis and interpretation, Manuscript writing, Revision of the manuscript, and Final approval of the manuscript.

Acknowledgements

We would like to thank to TÜBİTAK for financially supporting the Project (#117S421) and to Dr. Khalid Shah (Brigham and Women's Hospital, Harvard Medical School) for providing us toxin resistant resistant cells, plasmids and lentiviral vectors. We would also like to thank Birnur Sinem Karaoğlan (International School of Medicine, İstanbul Medipol University) for helping with manuscript formatting.

Funding

This work is a part of the project (#117S421) (N.K.) supported by TÜBİTAK.

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Received March 30, 2021
Revised May 19, 2021
Accepted May 23, 2021