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Targeting Interleukin-4 Receptor α with Hybrid Peptide for Effective Cancer Therapy

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Abstract

Interleukin-4 receptor alpha chain (IL-4R α) is highly expressed on the surface of various human solid tumors. We designed a novel hybrid peptide termed IL4R α -lytic peptide that targets the IL-4R α chain. The IL4R α -lytic peptide contains a target moiety to bind to IL-4R α and a cellular toxic lytic peptide that selectively kills cancer cells. The anticancer activity of the IL4R α -lytic peptide was evaluated *in vitro* and *in vivo*. It was found that IL4R α -lytic peptide has cytotoxic activity to cancer cell lines expressing IL-4R α , determined by quantitative real-time PCR. The IC₅₀ ratios of the lytic peptide to the IL4R α -lytic peptide correlated well with the expression levels of IL-4R α on cancer cells ($r=0.80$). In addition, IL4R α -lytic peptide administered by either intratumorally or intravenously significantly inhibited tumor growth in xenograft model of human pancreatic cancer (BXPC-3) in mice. These results indicate that the IL4R α -lytic peptide generated in this study has a potent and selective anticancer potential against IL-4R α positive solid cancers.

Introduction

By increasing knowledge of unique or overexpressed cell-surface antigens or receptors on tumor cells as targets, immunotoxin, one of the form of cancer therapy drug, has been developed over the last 3 to 4 decades. Immunotoxins are proteins that are composed of a target binding moiety (an antibody or growth factor that binds specifically to target cells) and a toxin moiety (a plant or bacterial toxin) (1). Some immunotoxins have been tested in clinical trails and they exhibited some efficacy in most tested patients (2, 3, 4, 5). An agent ONTAK that contains human interleukin-2 and truncated diphtheria toxin has been approved for use in cutaneous T-cell lymphoma (6).

However, there are concerns of immunogenicity and hepatotoxicity caused by the immunotoxins (7, 8). Moreover, due to their larger molecular sizes compared to chemical compounds or fragment antibody drugs, many immunotoxins might have difficulty in penetration into human tumor mass (6). To reduce immunogenicity caused by the immunotoxins, several approaches have been used for the design of immunotoxins, such as chemical modification with polyethylene glycol (PEGylation) or fusion with a single-chain Fv of an antibody (9, 10). PEGylation not only blocks immunogenicity but also prolongs half-life. But these immunotoxins still have larger

molecular weight and are rather difficult to produce in larger scale.

To overcome these problems, a new “hybrid peptide” drug, which has similar concept with immunotoxin but smaller molecular weight, has been developed (11). Anti-cancer hybrid peptide (type I) contains target-binding amino acids and toxic amino acid sequences. These molecules are chemically stable, small, and can be synthesized by simple peptide chemistry (12).

In the toxic part of the hybrid peptide, we have utilized a new lytic peptide (11), which is stable when combined with targeting peptide with less toxic to normal cell lines when compared to original lytic peptide composed of a 15-amino-acid diastereomer composed of D- and L-amino acids (13).

High-affinity interleukin-4 receptor (IL-4R) are highly expressed on the surface of various human solid tumors including renal cell carcinoma, melanoma, breast carcinoma, ovarian carcinoma, glioblastoma, AIDS-related Kaposi’s sarcoma, and head and neck squamous cell carcinoma (14, 15, 16, 17, 18, 19, 20). IL-4R-targeted protein-based immunotoxin was being tested in the clinic for the treatment of human solid tumors (3, 21, 22). The significance of expression of IL-4R on cancer cells still remains obscure. However, these receptors are able to mediate biological responses in cancer cells such as regulation of intercellular adhesion molecule-1 and major

histocompatibility complex antigen expression, inhibition of cell growth, and induction of apoptosis (23). The IL-4R system exists in three different types. Type I IL-4Rs are consisted of a major protein (IL-4R α) and the IL-2R γ chain (γ c) (24, 25). Type II IL-4Rs are composed of IL-4R α and IL-13R α 1 chains. Type III IL-4Rs express all three chains. The IL-4Rs in solid tumor cells are composed of IL-4R α and IL-13R α 1 chains (type II IL-4Rs) (26, 27, 28).

These results prompted us to design a new hybrid peptide targeting IL-4R α -overexpressing cancer cells, comprising of an IL-4R α -binding moiety and the cellular membrane-lytic moiety, termed IL4R α -lytic hybrid peptide. In this study, we examined the selective cytotoxicity of IL4R α -lytic hybrid peptide to cancer cells *in vitro*, and antitumor activity of the peptide *in vivo*.

5'-GCAGACGGACAACACGATACAG-3') or each of GAPDH primers (forward, 5'-GTCTTCACCACCATGGAGAAGGCT-3'; reverse, 5'-CATGCCAGTGAGCTTCCCGTTCA-3'). GAPDH was used as an internal control. PCR was carried out for 35 cycles at 94 °C for 30 sec, 60 °C for 30 sec, and 72 °C for 30 sec. PCR product was run in a 1% agarose gel for UV analysis.

Quantitative Real-time PCR analysis. Quantitative real-time PCR was carried out using SYBR Green Real-time PCR Master Mix kit (TOYOBO, Osaka, Japan) at Mx3000P Real-Time QPCR System (Stratagene, La Jolla, CA). Amplification was performed under the following conditions: 45 cycles of 95 °C for 15 sec, 60 °C for 15 sec, and 72 °C for 45 sec. The primers are same with RT-PCR analysis.

Annexin V analysis. Cells (PE and SU.86.86.) were cultured in 6-well plates for 15 h and then were treated for 2 h at 37 °C with or without lytic peptide alone or IL4R α -lytic peptide at 10 μ M. Cells were washed, collected, and the flow cytometry (Becton Dickinson, Mountain View, CA) analysis was performed using Annexin V-Fluorescein Staining kit (Wako, Osaka, Japan). Data were analyzed using CellQuest Software.

Cell cycle analysis. Cell cycle analysis was performed as described previously (29).

Briefly, SU.86.86. cells were seeded into 6-wells plate overnight. The cells were then treated with or without IL4R α -lytic peptide. Then the cells were collected, washed with PBS and fixed in ice-cold 70% ethanol at -20°C overnight. After washed twice with PBS, the cell pellet was resuspended in 0.25 mg/ml RNase A (Nacalai Tesque) for 30 min at 37°C and in 50 μ g/ml propidium iodide (Nacalai Tesque) for 30 min at 4°C. The cells were next analyzed using FACS Calibur flow cytometry and Cell Quest software (Becton Dickinson, Mountain View, CA).

Terminal deoxynucleotidyl transferase-mediated UTP end labeling (TUNEL) assay.

SU.86.86. cells were cultured in 6-well plate overnight. After incubation with or without IL4R α -lytic peptide, TUNEL assay was performed by MEBSTAIN Apoptosis Kit Direct (MBL) using flow cytometry according to the manufacture's instructions.

Antitumor activity of IL4R α -lytic in tumor xenografts *in vivo*. Six to 7-week-old

female athymic nude mice (BALB/c nu/nu) were obtained from SLC (Sizuoka, Japan).

Human breast tumors were established in nude mice by subcutaneous (s.c.) injection of 5×10^6 BXPC-3 or MDA-MB-231 cells in 150 μ l of PBS into the flank. After 5 days,

mice were randomized into three groups, and saline (control) or IL4R α -lytic peptide (2 or 5 mg/kg) was injected intratumorally (i.t.) or intravenously (i.v.) (50 μ l/injection) three times a week for 3 weeks. Tumors were measured with a caliper, and the tumor volume was calculated using the following formula: (length of the tumor) \times (width of the tumor)²/2 (11). The significance of differences between groups was determined by Student *t* test. *P* values of < 0.05 were considered statistically significant.

Toxicity assessment. For serum chemistry and organ toxicity, serum and tissue samples were obtained 1 day after last injection of the IL4R α -lytic hybrid peptide. Organs from these experimental animals were fixed with 10% formalin. Tissue sections (5 μ m) prepared from paraffin-embedded blocks were stained with H&E. Microscopy analysis was performed using Olympus DP25 microscopy (Tokyo, Japan).

Results

Design of IL4R α -lytic peptide. It is known that various solid tumor cells highly express type II IL-4Rs which is composed of IL-4R α and IL-13R α 1 chains (Fig. 1A) and IL-4R α chain binds IL-4 with high affinity (K_d 20 to 300 pM). Fig. 1B shows the structure in the contact interface between human IL-4 and IL-4R α . It was previously shown that five positively charged residues (K77, R81, K84, R85, and R88) and a neighboring residue (N89) are important for binding of human IL-4 to IL-4R α (30, 31, 32). In addition, it was also reported that in the murine IL-4, the main binding site is 79QRLFRAFR86 and the residues R80, R83 and R86 play a crucial role for binding to IL-4R α (33). Three arginine residues R81, R85 and R88 of human IL-4 may mimic those arginine residues of mouse IL-4 in binding to IL-4R α as shown in the alignment among human, bovine, murine, and rat IL-4 sequences (Fig. 1C). From these results we have designed an IL-4 peptide, 77KQLIRFLKRLDRN89, which includes the critical amino acids R81, R85 and R88. SPR analysis showed that the designed peptide can bind to recombinant IL-4R α with the K_d value of 2.90×10^{-4} M by BIACORE system (data not shown). We then produced IL4R α -lytic peptide, which contains lytic sequence (11) including three glycine as a spacer. Mutation analysis of IL4R α -binding peptide also showed that the sequence shown here was the best to achieve the cytotoxic activity

to IL4R α -expressing cancer cells, as assessed by WST-8 assay (data not shown).

Expression levels of IL-4R α in normal and cancer cell lines. Normal pancreatic cell line PE and seven cancer cell lines including BXPC-3 and SU.86.86. pancreatic cancer, KB head and neck cancer, T98G and A172 glioblastoma, H322 lung cancer, and MDA-MB-231 breast cancer were examined for mRNA expression of IL-4R α by RT-PCR analysis (Fig. 2A). It was found that the normal cell line PE did not express IL-4R α mRNA. On the other hand, six cancer cell lines expressed different levels of IL-4R α mRNA. To further compare the expression levels of IL-4R α mRNA in these cells, quantitative real-time PCR was carried out, and it was demonstrated that BXPC-3, SU.86.86., T98G, A172, H322, and MDA-MB-231 cell lines expressed high levels of IL-4R α . On the other hand, KB cells expressed low level of IL4R α , and mRNA of IL-4R α was not found in the normal PE cells (Fig. 2B).

Selective killing of cancer cell lines by IL4R α -lytic peptide. To assess the cytotoxic activity of IL4R α -lytic peptide, WST-8 assay was performed using normal and cancer cell lines treated with lytic peptide alone or IL4R α -lytic peptide. As shown in Fig. 2C, both lytic peptide and IL4R α -lytic peptide induced a concentration-dependent

cytotoxicity to BXPc-3 and SU.86.86. cancer cells. Less than 10 μ M dose of IL4R α -lytic peptide sufficiently induced more than 80% of cell death of BXPc-3 and SU.86.86.. Whereas, the same concentration of this peptide did not induce cell killing of normal cells (PE). These results suggest that IL4R α -lytic peptide has selective cytotoxic activity to distinguish between normal and cancer cells.

Enhancement of the IL4R α -lytic peptide-induced cytotoxicity correlates well with the expression levels of IL-4R α . The cytotoxic activity of lytic and IL4R α -lytic peptides is as shown in Table 1. The cytotoxic activity of hybrid peptide was enhanced when compared with that of lytic peptide alone. The IC₅₀ (peptide concentration inducing 50% inhibition of control cell growth) of IL4R α -lytic peptide improved 2.0-to 5.1-fold. We then examined whether the enhancement of cytotoxicity of IL4R α -lytic peptide was correlated with the expression levels of IL-4R α in the cells. The expression levels of IL-4R α in cells was correlated well with IC₅₀ ratio of lytic peptide to IL4R α -lytic peptide ($r=0.80$, data not shown), indicating the enhancement of cytotoxic activity due to the targeting (IL-4R α) moiety of hybrid peptide. These results suggest that the increase in cytotoxic activity depends on the expression levels of IL-4R α in cell.

IL4R α -lytic peptide induces rapid killing of cancer cells. We next examined the time course of IL4R α -lytic peptide to induce loss of viability of PE normal and BXPC-3 cancer cells. As shown in Fig. 3, 10 μ M of IL4R α -lytic peptide induced 50% of cancer cell death within 5 to 10 min, and 80% of cells were killed by this hybrid peptide after 1 h, but the same concentration of lytic peptide alone did not induce cytotoxic activity (Fig. 3A). On the other hand, neither lytic peptide alone nor IL4R α -lytic peptide did induce optimal cell killing to PE (Fig. 3B). These results suggest that IL4R α -lytic hybrid peptide can rapidly and selectively kill cancer cells.

Characterization of cancer cell death mechanism by IL4R α -lytic peptide. To reveal the mechanism of cancer cell death induced by IL4R α -lytic hybrid peptide, flow cytometry analysis was performed using annexin V. As shown in Fig. 4A, annexin V positive cells were found when 10 μ M of IL4R α -lytic peptide was added to SU.86.86 cells. The percentage of annexin V positive and PI negative cells (19.78%, lower right region) of SU.86.86 cells treated with IL4R α -lytic peptide was higher than that of the control (11%), and the percentage of annexin V and PI positive cells (59.54%) was remarkably higher than the control (3.67%). However, the percentage of annexin V

positive cells of SU.86.86 cells treated with lytic peptide was not significantly different from that of the control. Treatment of PE cells with lytic peptide alone or IL4R α -lytic peptide (10 μ M) for 2 h did not increase annexin V positive cells at all. The ratio of annexin V positive cell percentage by either lytic peptide alone or IL4R α -lytic peptide when compared to untreated normal cells (PE) as control was 1.23 (PE) and 5.44 (SU.86.86.) (lytic peptide alone), and 1.77 (PE) and 25.50 (SU.86.86.) (IL4R α -lytic peptide), respectively (Fig. 4B). To further clarify the characterization of cancer cell death induced by IL4R α -lytic peptide, cell cycle analysis and TUNEL assay were performed. When SU.86.86. cells were treated with IL4R α -lytic peptide for 16 h, the percentage of sub-G1 was increased from 6.78% to 19.17%, and the mean fluorescent intensity (MFI) was increased from 2.19% to 42.54%, compared to untreated cells (Supplementary Fig. S1A). On the other hand, there was no significant difference on the percentage of sub-G1 and the value of MFI between treated and untreated cells after 1 h exposure to the same concentration of IL4R α -lytic peptide (Fig 4C). Furthermore, it was found that cell viability was quickly decreased within 1 h after the treatment with IL4R α -lytic hybrid peptide (Supplementary Fig. S1B). Taken together with these results, it is suggested that the increases in the percentage of sub-G1 population and the MFI value of TUNEL assay were induced by secondary effect after rapid cancer cell death

with IL4R α -lytic hybrid peptide, and the apoptotic cell death induced by IL4R α -lytic hybrid peptide is not primary.

Antitumor activity of IL4R α -lytic peptide *in vivo*. *In vitro* experiments indicated that IL-4R α positive cancer cell lines are highly sensitive to IL4R α -lytic peptide. To assess antitumor activity of this hybrid peptide *in vivo*, nude mice received s.c. injections of 5×10^6 BXPC-3 cells. The efficacy of IL4R α -lytic peptide, when administered by different routes, was evaluated in these mice. I.t. administration of IL4R α -lytic peptide (2 or 5 mg/kg, 3 times a week) significantly inhibited tumor growth. As shown in Fig. 5A (left graph), tumors in saline treated control mice grew aggressively, reached 979 mm³ by day 59. On the other hand, animals treated with IL4R α -lytic peptide showed significant tumor regression at both dosages, the mean tumor sizes were 553 mm³ (2 mg/kg) and 234 mm³ (5 mg/kg) in the treated mice on day 59. I.v. treatment also showed antitumor activity. As shown in Fig. 5A (right graph), the effect of IL4R α -lytic peptide was clearly dose-dependent. Two mg/kg dose of the treatment was less effective against BXPC-3 tumor growth. The mean tumor volume was 752 mm³ on day 59, which is smaller than control tumor volume (1182 mm³, $P < 0.05$). Five mg/kg dose of IL4R α -lytic peptide exhibited superior antitumor activity. The mean tumor volume of

treated tumors was 402 mm³, which is significantly smaller compared with the control tumor at day 59 ($P < 0.05$).

To preliminary assess peptide drug-related organ toxicities, analyses of complete blood counts and blood serum chemistry, and pathology experiments of major organs (including liver, and kidney) were performed using animals receiving i.t. or i.v. administration of IL4R α -lytic peptide. Samples were obtained one day after the last injection of drug. In blood examination, animals treated with 5 mg/kg dose of IL4R α -lytic peptide by i.v. route showed a minor decline of white blood cell count compared with the control mice. No other abnormality was observed in mice treated with IL4R α -lytic peptide either by i.t. or i.v. routes (data not shown). Microscopic analysis showed no abnormal changes in the major organs tissues obtained from the mice treated with IL4R α -lytic either by i.t. or i.v. routes (Fig. 5B shows pictures of liver and kidney). Remarkable loss of body weight was not observed.

On the mouse xenograft model of human breast cancer MDA-MB-231, IL4R α -lytic hybrid peptide also showed effective antitumor activity when administered by i.t. or i.v. injection (Supplementary Fig. S2A). No abnormal changes were observed in the major organs tissues obtained from the mice treated with IL4R α -lytic hybrid peptide (Supplementary Fig. S2B). In addition, no remarkable loss of body weight was

observed.

These results indicate that the IL4R α -lytic hybrid peptide selectively targets to cancer cells inducing tumor regression without unexpected organ toxicities.

Discussion

It has been reported that several peptides composed of L-amino acids exhibited cytotoxic activity against cancer cell lines *in vitro* (34, 35), however, most of these L-amino acid-based peptides also affected normal cells, limiting clinical usage. Moreover, some of these L-amino acids peptides failed to exhibit desirable antitumor activity *in vivo* because these peptides lose cytotoxic activity in serum in the body circulation due to enzymatic degradation and binding to serum components (36). Supporting these reports, in the current study we also found that both a lytic peptide which is entirely composed of L-amino acids and a hybrid peptide composed of IL-4R α binding moiety and the lytic moiety composed of L-amino acids, killed normal cell lines at a low concentration *in vitro*, and failed to show antitumor activity *in vivo* (data not shown). Papo N *et al.* developed a novel lytic peptide composed of DL-amino acids, which selectively killed cancer cells *in vitro* and *in vivo* (13). However, since we found that this lytic sequence was not suitable to combine with targeting moiety, we modified the DL-amino acids sequence to appropriately induce modest cancer cells killing, with less toxicity to normal cells in a lower concentration (11). Similar to the lytic peptide previously reported, the new lytic sequence has positive charge and binds to negatively charged membranes (37) and subsequently lyses them (38). It is known that the outer

membrane of cancer cells contains a slightly more negatively charged phosphatidylserine than that of normal cells (39). This fact probably, at least partly, contributes to the selectively killing cancer cells of lytic peptide.

By using peptide phage display and molecular modeling, Yao G *et al.* have showed that in murine the amino acid residues spanning from 76 to 86 (QRLFRAFR) especially the residues R80, R83, and R86 play a crucial role in binding to the IL-4R α chain (39). It has also been shown that residues K77, R81, K84, R85, R88, and N89 are important for binding of human IL-4 to IL-4R (31, 32]. Three arginine residues R83, R85, and R88 on human IL-4 may mimic arginine residues R80, R83, and R86 on mouse IL-4 in binding to IL-4R α . Taken together, we hypothesize that 77KQLIRFLKRLDRN89 peptide, the IL-4R α moiety designed in this study, can specifically bind to IL-4R α on cells. As shown in Fig. 2, IL4R α -lytic peptide exhibited enhanced cytotoxic activity to cancer cells expressing IL-4R α *in vitro* when compared with lytic peptide alone. The enhancement of the cytotoxic activity against cancer cells depended on the expression levels of IL-4R α on the cells. Normal cell PE without expressing IL-4R α is found not sensitive to IL4R α -lytic peptide. These results suggest that the binding moiety peptide designed in this study can specifically bind to IL-4R α in cells.

Although increase in the percentage of sub-G1 population and TUNEL positive cells were found after 16 h treatment with IL4R α -lytic peptide, these positive cells were not almost found after 1 h treatment with this peptide (Fig. 4C and Supplementary Fig. S2A). Since IL4R α -lytic peptide quickly induced cancer cell death (Fig. 3 and Supplementary Fig. S2B), it is suggested that these apoptotic positive cells were induced by secondary effect, however, the detail mechanism of cancer cell death induced by IL4R α -lytic hybrid peptide is still obscure.

It was also found that IL4R α -lytic peptide exhibited high cytotoxic activity against cancer cells expressing IL-4R α *in vitro* (Fig. 2C), and that i.t. administration of this peptide dramatically inhibited the growth of pancreatic cancer BXPC-3 (Fig. 5A) or breast cancer MDA-MB-231 (Supplementary Fig. S2A) tumors *in vivo*. In addition, i.v. administration of this peptide induced dose-dependent regression of BXPC-3 (Fig. 5A) or MDA-MB-231 (Supplementary Fig. S2A) tumors. We assume that because i.t. administration hold the drug locally at higher concentration in tumor, the antitumor effect exhibited was superior. No specific toxicity was found by either i.t. or i.v. administration. Taken together, IL4R α -lytic peptide might be a potent anticancer drug to IL-4R α -expressing solid tumor, under the condition of local administration or the systemic administration in combination with the suitable drug delivery system.

In conclusion, in this study, we described the IL4R α -lytic hybrid peptide targeting IL-4R α in cancer cells. Further analyses to this drug including cytotoxic mechanisms, detailed safety profiles in animals, and justification of the appropriate usage in clinic will be necessary. These researches are currently ongoing in our laboratory.

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Table 1. Cytotoxic activity of peptide to various cell lines and IL4R α expression

Cell lines	IC ₅₀ (μ M)		IC ₅₀ Ratio Lytic/IL4R α -lytic	IL4R α Relative Expression*
	lytic	IL4R α -lytic		
	Mean \pm SD	Mean \pm SD		
Normal cells				
PE	70.3 \pm 2.4	35.2 \pm 1.7	2.0	N.D.
Cancer cells				
BXPC-3	37.1 \pm 0.7	6.8 \pm 0.3	4.7	6.0
SU.86.86.	28.0 \pm 0.5	7.5 \pm 0.4	3.7	7.3
KB	37.4 \pm 0.9	13.2 \pm 0.8	2.8	1.0
T98G	77.3 \pm 1.7	18.5 \pm 0.7	4.2	4.8
A172	30.5 \pm 0.9	6.8 \pm 0.4	4.5	6.3
H322	18.5 \pm 0.4	3.6 \pm 0.5	5.1	8.0
MDA-MB-231	27.1 \pm 1.5	5.7 \pm 0.4	4.8	3.4

*The relative expression was determined by quantitative real-time PCR.

N.D., Not detected

Figure legends

Figure 1 Structures of human IL-4 and IL-4R α and aligned sequences of mature IL-4.

(A) Schematic model of IL-4R on solid tumor cells. (B) Structure in the contact interface of human IL-4 and IL-4R α . Significant residues R81, R85, and R88 of IL-4 for the binding to IL-4R α are indicated as red, magenta and green color. The information about structure was obtained from Protein Data Bank (1iar), and stick model is shown using Ras Mol software. (C) Aligned sequences of mature IL-4. The sequences for mature IL-4 were aligned using the program Clustalw. The red letters are the important sequences for human IL-4 binding to IL-4R α . The blue letters are the critical residues of murine IL-4 binding to IL-4R α .

Figure 2 The expression levels of IL-4R α on normal and cancer cell lines and the cytotoxic activity of IL4R α -lytic hybrid peptide. Total RNA from normal (PE) and cancer (BXPC-3, SU.86.86., KB, T98G, A172, H322, and MDA-MB-231) cell lines were reverse transcribed to cDNA, and then assessed by PCR (A) or real time quantitative PCR (B) using IL-4R α -specific primers. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) served as an internal control. (C) Cytotoxic activity of IL4R α -lytic hybrid peptide to normal and cancer cell lines. The data was expressed as a

percentage of the untreated control cells (% of control). The experiments were performed at least three times.

Figure 3 Rapid killing of cancer cells by IL4R α -lytic hybrid peptide. BXPC-3 (A) or PE (B) cells were treated with IL4R α -lytic hybrid peptide (10 μ M) (*black columns*) or lytic peptide alone (10 μ M) (*white columns*) for indicated time periods. Cell viability was determined using WST-8. The results are represented as means \pm SD (bars).

Figure 4 Characterization of cancer cell death mechanism by IL4R α -lytic hybrid peptide. (A) Annexin V assay. PE or SU.86.86. cells treated with or without lytic or IL4R α -lytic peptide (10 μ M) for 2 h were analyzed by flow cytometry for annexin V and propidium iodide (PI) staining. The percentage of cells in each quadrant is as indicated. (B) Fold activation of cell death compared to untreated PE cells. The results were represented as the fold increase of untreated dead PE cells (Annexin V positive cells) as control. (C) Cell cycle and TUNEL assay. SU.86.86. cells were treated with or without IL4R α -lytic peptide (3 μ M) for 1 h. The percentage of cells in the sub-G1 population of the cell cycle, and the intensity of TUNEL signal (mean fluorescence intensity: MFI) were determined by flow cytometry.

Figure 5 Antitumor activity of IL4R α -lytic hybrid peptide *in vivo*. BXPC-3 pancreatic cancer cells were implanted subcutaneously into athymic nude mice. Intratumoral (i.t.; A, left graph) or intravenous (i.v.; A, right graph) injection of either saline or IL4R α -lytic hybrid peptide (2 or 5 mg/kg) was provided 3 times per week from the 5th day for 3 weeks as indicated by the arrows. Each group had six animals (n=6). Data are expressed as mean \pm SD (bars). (B) Liver and kidney organs obtained from the mice treated as above were stained with H&E. Images (400 \times magnification) were obtained using light microscopy. Scale bars, 20 μ m.

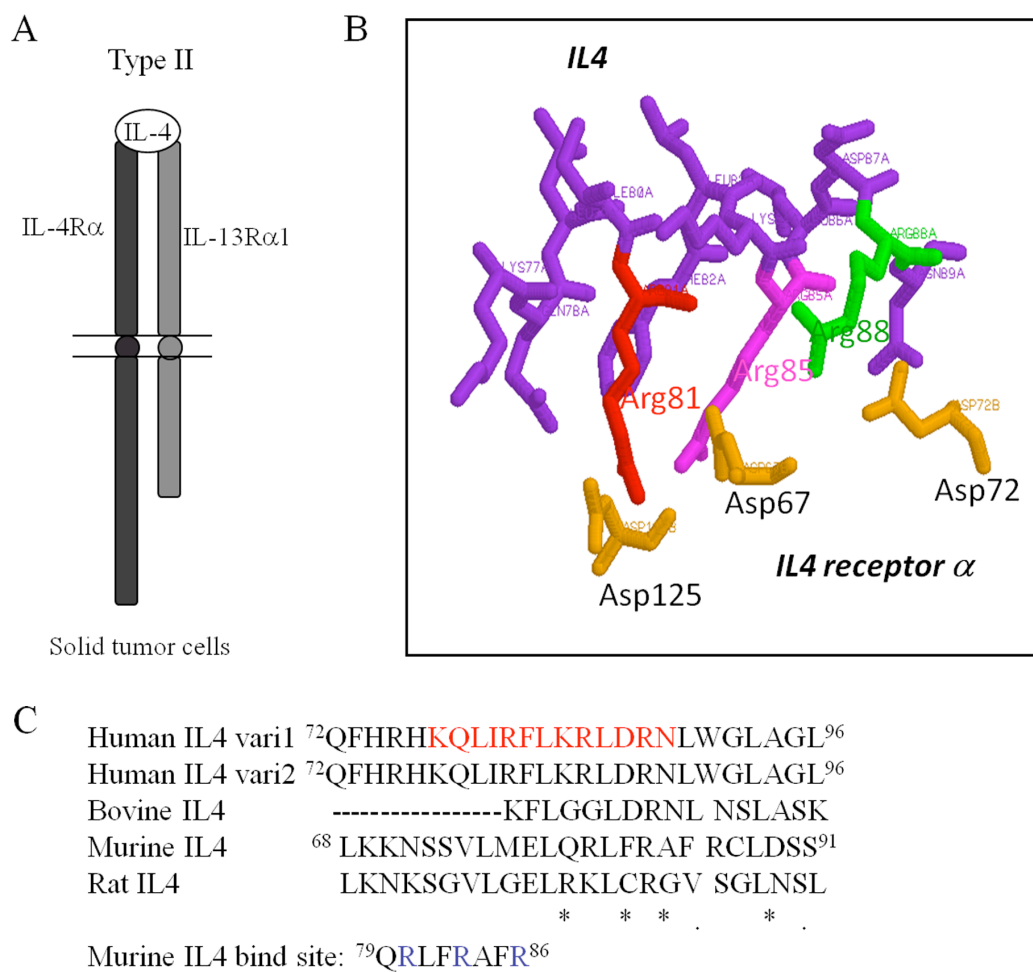


Figure 1 (A-C) Yang *et al.*

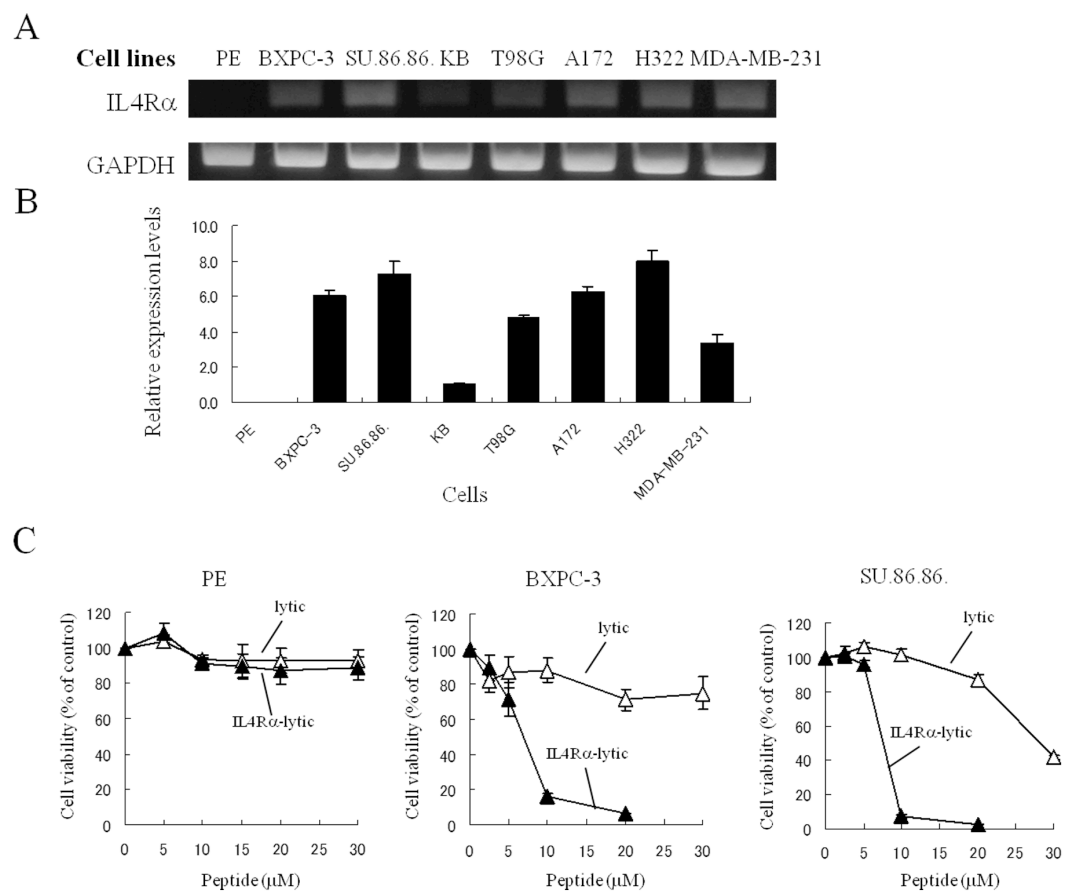


Figure 2 (A-C) Yang *et al.*

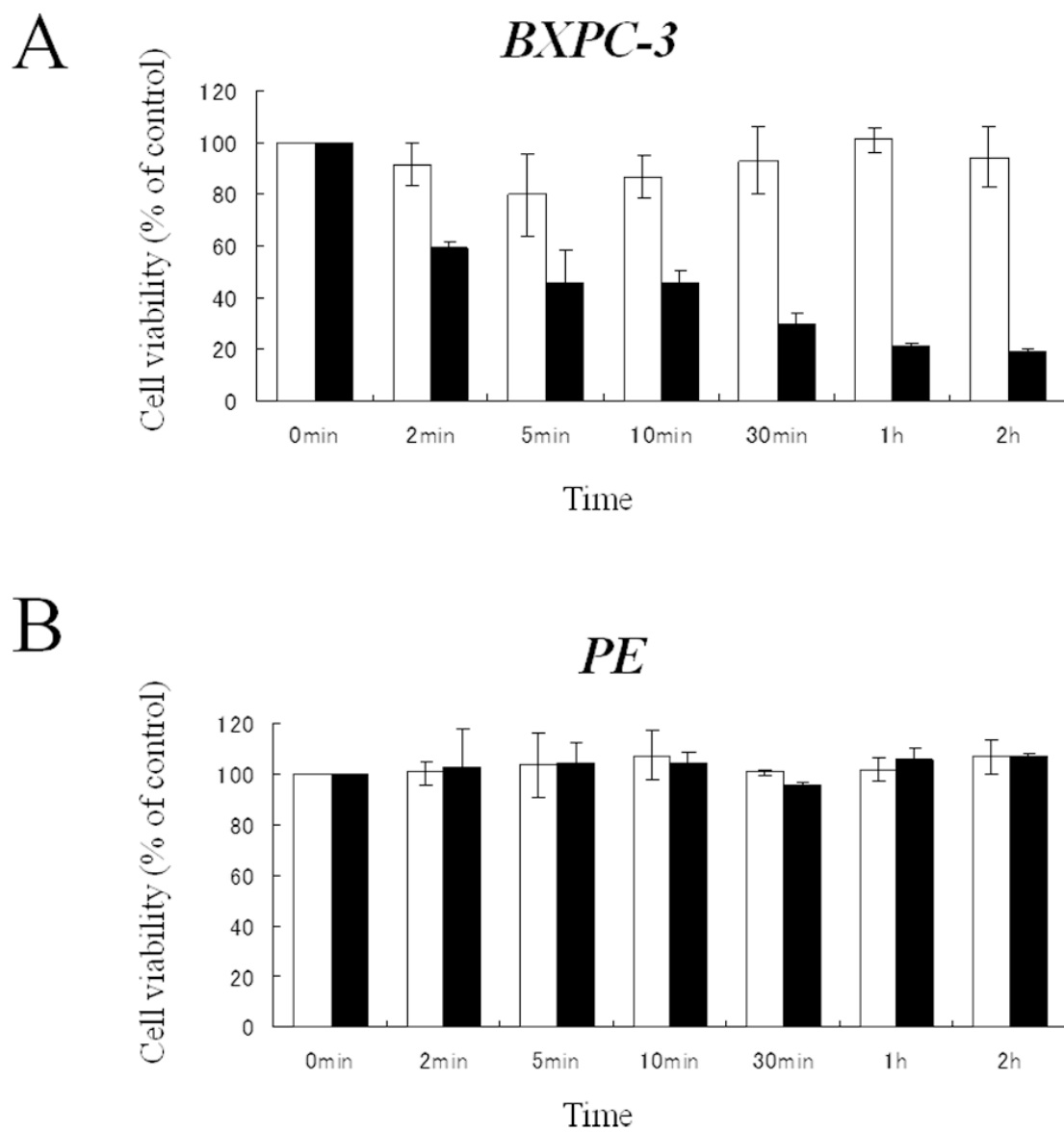


Figure 3 (A and B) Yang *et al.*

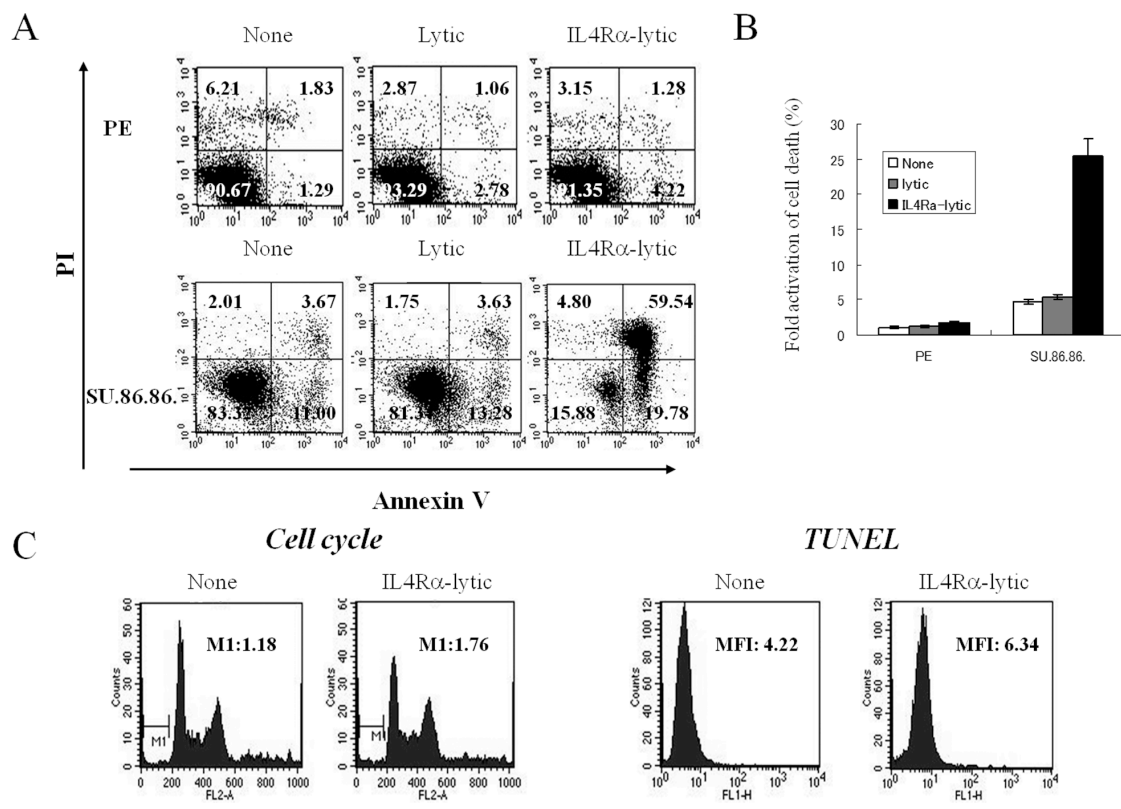


Figure 4 (A-C) Yang *et al.*

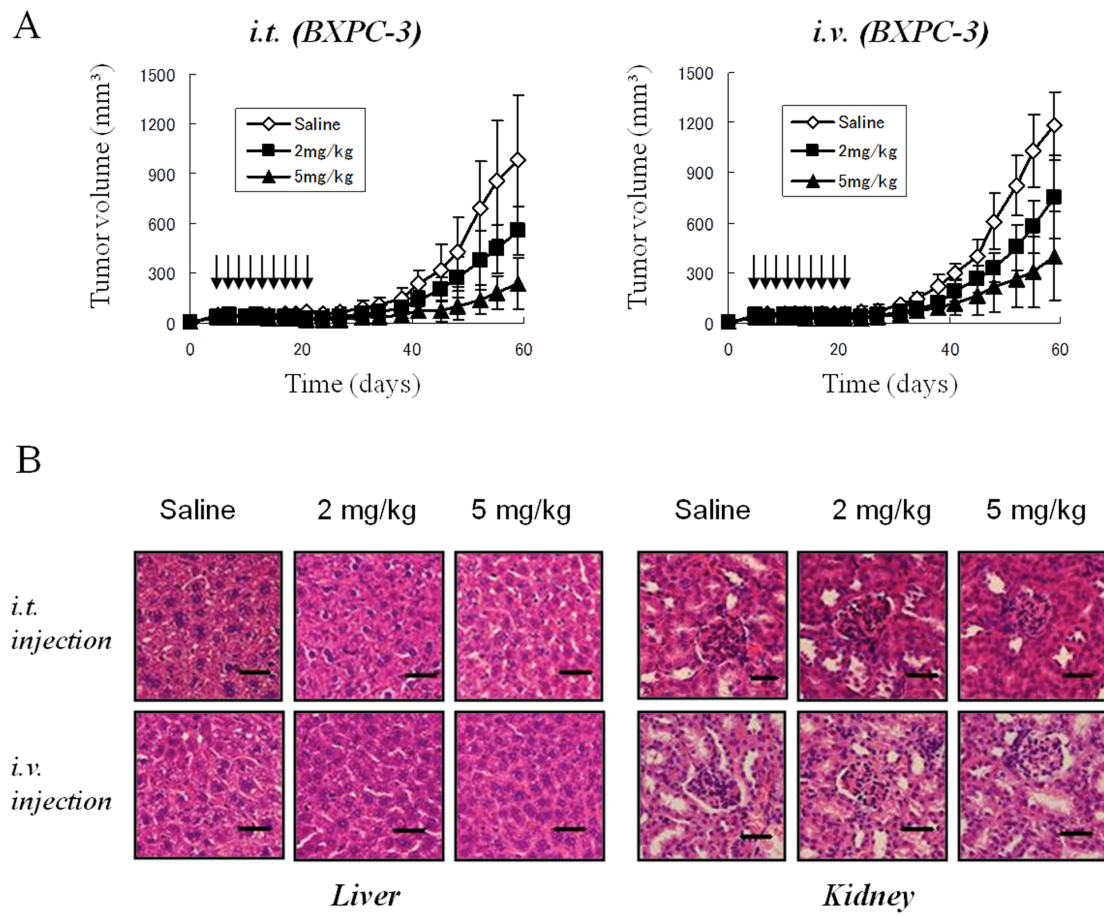


Figure 5 (A and B) Yang *et al.*

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