Antitumor effect of a transducible fusogenic peptide releasing multiple proapoptotic peptides by caspase-3

Mi-Kyung Kwon,1 Ju-Ock Nam,1 Rang-Woon Park,1,3 Byung-Heon Lee,1,3 Jae-Yong Park,1,2,3 Young-Ro Byun,5 Sang-Yoon Kim,6 Ick-Chan Kwon,4,7 and In-San Kim1,3

1Department of Biochemistry and Cell Biology, Cell and Matrix Research Institute, 2Department of Internal Medicine, 3Advanced Medical Technology Cluster for Diagnosis and Prediction, and 4KIST Regional Laboratory, The Advanced Medical Technology Cluster for Diagnosis and Prediction, School of Medicine, Kyungpook National University, Daegu, Korea and 5College of Pharmacy, Seoul National University; and 6Departments of Nuclear Medicine and Otolaryngology, Asian Medical Center, University of Ulsan College of Medicine; and 7Biomedical Research Center, Korea Institute of Science and Technology, Seoul, Korea

Abstract

We have designed a novel peptide, TK3, composed of three functional domains, a protein transduction domain, a TAT followed by three tandem repeats of a proapoptotic peptide, and a caspase-3 cleavage site, (KLAKLAK)2-DEVD. TK3 was able to transduce into cells and then activate caspase-3, which in turn cleaved TK3 to release additional (KLAKLAK)2 peptides. (KLAKLAK)2 was well transduced by TAT into tumor cells and was able to induce apoptosis in vitro and in vivo. TK3 also induced apoptosis and inhibited angiogenesis in endothelial cells. Further, direct injection of TK3 into established B16F10 melanoma tumors in C57BL/6 mice resulted in almost complete inhibition of the tumor growth. These results suggest that TK3 could be beneficial for the treatment of accessible tumors and used as an adjuvant for cancer therapy. [Mol Cancer Ther 2008;7(6):1514–22]
Construction of Expression Plasmids

pTK was constructed in the following manner to express the basic domain (amino acids 49-57) of HIV-1-TAT as a fusion protein with KLAKLAKLAKLAKLAK [(KLAKLAK)2]. First, a double-stranded oligonucleotide encoding the nine amino acids of TAT, RKKRRQRRR, was cloned into pET30a(+) vector (Novagen) within the NdeI and EcoRV sites to generate pTAT. Next, pcDNA-(KLAKLAK)2-Myc was generated by inserting a double-stranded oligonucleotide encoding GG-(KLAKLAK)2 into pcDNA3.1/myc-His(-)A (Invitrogen) within the NheI and HindIII sites. The (KLAKLAK)2-Myc sequence was then amplified by PCR (sense primer: 5'-AAAACTGCAGGCTAGCGGAGGTAAG-3' and antisense primer: 5'-AAAACTCGAGATTCCAGACCTCTTC-3') from pcDNA-(KLAKLAK)2-Myc and subcloned into pTAT within the PstI and XhoI sites to generate pTK. Next, pmTK was generated by replacing (KLAKLAK)2 with DLSLARLATARLAI. The complete enhanced green fluorescent protein (GFP) gene sequence was then amplified from plasmid pEGFP-C1 (Clontech) by PCR (sense primer: 5'-AAAAGAGCTCCCCTAGCGC-TAACC-3' and antisense primer: 5'-AAACTCGAGGATCTGAGTCCGGACTT-3') followed by subcloning into pTK, pET28a(+) (Novagen), and pmTK within the SacI and XhoI sites to generate pTK-GFP, pEGFP, and pmTK-GFP, respectively. A double-stranded oligonucleotide encoding (KLAKLAK)2-DEVD-GG was then inserted into pTAT-GFP within the BamHI and SacI sites to construct pTK1-GFP followed by the tandem insertion of two more copies of (KLAKLAK)2-DEVD-GG into pTK1-GFP within the BamHI and SacI sites one by one to construct pTK3-GFP.

Expression and Purification of Fusion Proteins

The fusion proteins were expressed in the BL21 (DE3) pLysS (Novagen) bacterial strain and then induced with 1 mmol/L isopropyl-β-D-thiogalactopyranoside. After bacterial cells were harvested and sonicated with binding buffer [20 mmol/L Tris-HCl (pH 8.0), 500 mmol/L NaCl, and 5'-AGCTTGGATCCACCTCCATCTCATCC-TTCGCGAGCTTCGCGAGCTTCGCGAGCTTCGCGAGCTTC-3']. Restriction enzyme sites that were incorporated to facilitate ligation are italicized.

**Figure 1.** Diagram of fusogenic peptides and visualization of TK-GFP transduced into cells. **A,** TK is composed of TAT-(KLAKLAK)2, whereas mTK is composed of TAT-(DLSLARLATARLAI). GFP is composed of only GFP without TAT. **B,** fluorescence microscopy of cells treated with fusogenic peptides. CHO and B16F10 cells were treated with 1.5 μmol/L GFP (**a**), TK-GFP (**b**), and mTK-GFP (**c**) for 2 h. Top, CHO cells (∼630); bottom, B16F10 cells (∼400). **C,** CHO cells were treated with various concentrations of TK-GFP, mTK-GFP, and GFP for 2 h, and the presence of transduced proteins in cells was then analyzed by Western blot using an anti-His horseradish peroxidase.
5 mmol/L imidazole (pH 7.9), 8 mol/L urea, 1 mmol/L phenylmethylsulfonyl fluoride, and 0.5 mmol/L DTT], the suspensions were clarified by centrifugation (12,000 rpm for 10 min at 4°C) and then subjected to purification on a Ni-NTA column (Qiagen) under denatured conditions. After washing by the stepwise addition of increasing imidazole concentrations to remove the high background of contaminating bacterial proteins, the target peptides were eluted using an elution buffer [20 mmol/L Tris-HCl, 500 mmol/L NaCl, 300 mmol/L imidazole (pH 7.9), and 8 mol/L urea] followed by desalting with a disposable PD-10 Sephadex G-25 column (Amersham Biosciences). The fusion proteins were used immediately after purification.

**Cell Culture**

Chinese hamster ovary cells (CHO; KCLB 10061) were cultured at 37°C and 5% CO₂ in MEM-a supplemented with 10% fetal bovine serum (FBS; Life Technologies). B16F10 mouse melanoma cells and human breast cancer cells (MCF-7) were cultured in RPMI 1640 (Life Technologies) supplemented with 10% FBS. Human umbilical vein endothelial cells (HUVEC) were isolated from umbilical cords by collagenase treatment and cultured in M199 medium (Sigma-Aldrich) supplemented with 20% FBS.

**Analysis of Transduced Cells by Fluorescence Microscopy**

Cells grown on coverslips in 24-well culture plates to 70% to 80% confluency were treated with the indicated concentrations of proteins. After 2 h of incubation, the cells were washed three times with PBS and then fixed in methanol/acetone (1:1) for 5 min at room temperature. The cells were then washed again with PBS and a solution of SlowFade solution (Molecular Probe) was added. A coverslip was overlaid and the fluorescence was analyzed using a fluorescence microscope (Zeiss Axioplan2; Carl Zeiss).

**Western Blot Analysis**

CHO cells (2 x 10⁵ per well) grown in six-well culture plates were treated with the indicated concentrations of proteins. After 2 h of incubation, the cells were harvested, washed three times with cold PBS (pH 7.4), and then resuspended in lysis buffer [20 mmol/L Tris-HCl, 137 mmol/L NaCl, 2 mmol/L EDTA, 2 mmol/L Na₃VO₃, 10% glycerol, 1% Triton X-100, and 1 mmol/L phenylmethylsulfonyl fluoride]. After centrifugation (12,000 rpm for 10 min at 4°C), the supernatant (30 μg protein/lane) was separated by electrophoresis on a 12% SDS-polyacrylamide gel and then transferred onto a nitrocellulose membrane. The membrane was then blocked with TBS-T (50 mmol/L Tris-HCl, 150 mmol/L NaCl, and 0.1% Tween 20) containing 3% skim milk for 1 h and incubated with horseradish peroxidase–linked anti-His antibody (Invitrogen) for 16 h at 4°C followed by detection with enhanced chemiluminescence reagent (Amersham Biosciences). Anti-β-actin (Sigma) and horseradish

---

**Figure 2.** TK inhibits cell growth and induces apoptosis. A, CHO, B16F10, and MCF-7 cells were treated with different concentrations of TK or mTK ranging from 0.5 to 15 μmol/L and then incubated for 3 h at 37°C. The cell viability was then assessed using a MTT assay. B, for the cell proliferation assay, CHO and B16F10 cells were treated with concentrations of TK or mTK ranging from 0.5 to 15 μmol/L for 3 h followed by incubation with BrdUrd for 20 h at 37°C. BrdUrd incorporation assay was then done. Columns, mean of triplicate wells; bars, SE. These experiments were repeated three times.

C, CHO and B16F10 cells were treated with 10 μmol/L TK (black peak) or mTK (gray peak) for 6 h. Cells were then stained with Annexin V-FITC for 20 min and monitored by fluorescence-activated cell sorting analysis. Negative control cells (white peak) were treated with PBS. Representative of two separately conducted experiments. *, P < 0.05; **, P < 0.01; ***, P < 0.001, versus untreated control.
peroxidase–labeled anti-mouse immunoglobulin (Santa Cruz Biotechnology) were used. Protein concentrations were determined according to the manufacturer’s instructions (Bio-Rad).

**Cell Proliferation Assay**

The measurement of viable cell numbers was carried out using a mitochondrial reduction activity assay (14). Briefly, cells (3 × 10^3 per well) were grown in 96-well culture plates overnight, at which time the medium was replaced with fresh medium containing 2% FBS. The cells were then treated with TK or mTK at concentrations ranging from 0.5 to 15 μmol/L for 3 h. In the case of TK1 or TK3, cells were treated for 24 h with concentrations ranging from 0.2 to 2 μmol/L. After incubation, 0.5 mg/mL 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; 50 μL) solution (Sigma) was added to each well. Cells were then lysed with DMSO and quantified by measuring the absorbance at 570 nm using a microplate reader 550 (Bio-Rad). A bromodeoxyuridine (BrdUrd) incorporation assay was then done as described previously (15). Briefly, cells were incubated in the presence or absence of TK or mTK in fresh medium containing 2% FBS for 3 h. After replacing the medium with fresh medium, BrdUrd was added and incubated for 20 h. The amount of incorporated BrdUrd was then assessed using a Cell Proliferation Assay Kit (Oncogene) following the manufacturer’s instructions.

**Figure 3.** TK-GFP transduces into tumors and inhibits tumor growth in vivo. B16F10 melanomas (∼1,000 mm^3) grown in C57BL/6 mice were injected with 100 μL of 20 μmol/L TK-GFP or GFP. After 30 min, the tumors were excised, cryosectioned, and observed by microscopy. A, fluorescence microscopy of tumors treated with GFP (a-c) or TK-GFP (d-f; ×630). B, serial z sections of 20 μmol/L TK-GFP-treated tumors (0.5 μm) observed using a confocal microscope (×1,000). C, B16F10 melanomas grown in C57BL/6 mice were injected with 50 μL of 120 μmol/L TK (n = 9), mTK (n = 8), or PBS (n = 10) daily for 8 consecutive days. The tumor size was measured everyday, and the tumor volume was then calculated. ***, P < 0.001, versus PBS-treated control. D, on day 8, the mice were sacrificed and their tumors were excised, cryosectioned, and stained. Representative terminal deoxynucleotidyl transferase–mediated nick end labeling staining is shown for three groups (×400).
Apoptosis Assay

Cells (2 \times 10^5 per well) were grown in six-well culture plates overnight and then incubated on plates in the presence of 10 \mu M TK or mTK in fresh medium containing 2% FBS for 6 h followed by labeling with FITC-conjugated Annexin V (Annexin V-FITC; Santa Cruz Biotechnology) for 20 min in the dark. Cells were then washed and analyzed by fluorescence-activated cell sorting at 488 nm in a FACSCalibur flow cytometry system (BD Biosciences) equipped with a 5 W argon laser.

Assay for Protein Cleavage by Caspase-3

To study the cleavage of TK3 by caspase-3, 1 \mu g TK3-GFP was incubated with various amounts of recombinant caspase-3 (Upstate) for 60 min or with 50 ng caspase-3 for various time periods in 6 mmol/L Tris-HCl (pH 7.5), 1.2 mmol/L CaCl_2, 5 mmol/L DTT, 1.5 mmol/L MgCl_2, and 1 mmol/L KCl. Reaction mixtures (17 \mu L/lane) were then separated by SDS-PAGE and transferred onto a nitrocellulose membrane followed by immunoblotting with horseradish peroxidase–linked anti-His antibody. Arrows, TK3-GFP protein (38.4 kDa) and its cleaved products (34.4, 32.18, and 30 kDa).

D, TK3-GFP protein (2 \mu M/L) was incubated with B16F10 cells at 37°C for 2 h. As a negative control (lane 2), the same amount of TK3-GFP protein was incubated without cells at 37°C for 2 h. Cell lysate and TK3-GFP protein were then analyzed by SDS-PAGE and immunoblotted with an anti-His antibody. Arrow, cleaved band of TK3-GFP.

Caspase-3 Assay

The caspase-3 assay of cell lysates was conducted as described previously. Briefly, B16F10 cells were grown to confluency and then treated with various concentrations of TK1 or TK3 for 3 h. Both the cells and the supernatants were centrifuged (1,500 rpm at 4°C), washed three times with cold PBS (pH 7.4), and then resuspended in lysis buffer. The lysates were then centrifuged at 12,000 rpm for 10 min at 4°C, and the supernatants were subjected to immunoblotting with an anti-caspase-3 antibody (Cell Signaling) and with a goat anti-rabbit horseradish peroxidase secondary antibody (Santa Cruz Biotechnology). The same membrane was then stripped and reprobed using a monoclonal anti-β-actin antibody (Sigma).

In vitro Angiogenesis Assay

An in vitro endothelial tube formation was done as described previously (16). Briefly, 100 \mu L Matrigel (Chemicon) was added to each well in 96-well culture plates and allowed to polymerize. HUVEC suspension (4 \times 10^5/mL; 100 \mu L) was then added to each well coated with Matrigel. After incubation for 2 to 3 h at 37°C, the cells were treated with the indicated concentrations of proteins for 3 to 5 h at 37°C. The cells were then photographed, and the number of branch points in five fields was counted (×100) for each sample.

CD31 Immunostaining

The intratumoral microvessel density was analyzed on sections of paraffin-embedded B16F10 tumor using a rat anti-mouse CD31 monoclonal antibody (BD Biosciences). Immunoperoxidase staining was conducted using the Vectastain avidin-biotin complex Elite reagent kit (Vector Laboratories). Sections were counterstained with hematoxylin. Microvessel density was assessed initially by scanning the tumor at low power followed by identification of eight areas containing the maximum number of discrete microvessels and by counting the individual microvessels under low magnification (×100).

In vivo administration

B16F10 cells (1 \times 10^5) were injected s.c. into 6 week-old male C57BL/6 mice in each flank. When 100 to 200 mm^3 tumors were observed, 50 \mu L of 120 \mu M/L TK, mTK, or PBS was injected into the tumors once a day for 7 days. Each injection was directed at multiple sites within the
using a fluorescence microscope and a confocal inverted microscope (Leica DM IRB; Leica Microsystems). All experiments were done in accordance with recommendations for the proper use and care of laboratory animals.

**Statistical Analysis**

All values are expressed as mean ± SE. The statistical significance of differential findings between experimental and control groups was determined using a Student’s *t* test. *P* < 0.05 was considered statistically significant.

**Results**

**Transduction of the Fusion Protein into Cells**

We generated constructs that expressed TAT fusion proteins in bacterial cells. TK contains nine amino acid residues of HIV-1-TAT followed by (KLAKLAK)_2, a proapoptotic peptide. In mTK, a non-α-helix-forming peptide, DSLRALARLAI, was used instead of (KLAKLAK)_2. GFP was linked with TK (TK-GFP) or mTK (mTK-GFP) to monitor the transduction of TAT fusion peptides (Fig. 1A). CHO cells were then treated with TK-GFP, mTK-GFP, or GFP for 2 h, after which TK-GFP and mTK-GFP were readily detected in the cells by fluorescence microscopy (Fig. 1B, *b* and *c*, *top*). Although TK-GFP and mTK-GFP could transduce the cells, GFP that did not contain TAT could not (Fig. 1B, *a*). B16F10 cells were also susceptible to TK-GFP or mTK-GFP transduction, as expected (Fig. 1B, *b* and *c*, *bottom*). Western blot analysis showed that TK-GFP transduction occurred in a concentration-dependent fashion and that mTK-GFP was also transduced into cells (Fig. 1C).

**TK Induces Apoptosis In vitro**

The proapoptotic peptide, (KLAKLAK)_2, has been known to disrupt mitochondrial membranes (1). Therefore, to determine whether transduced TK retains its proapoptotic activity, we tested cell viability using a MTT assay. Considerable cell death was observed in CHO, B16F10, and MCF-7 cells (Fig. 2A) that were treated with TK for 3 h, which lead to a decrease in the percent viability when compared with cells treated with mTK as a negative control. We also measured cell proliferation using a BrdUrd incorporation assay and found that the proliferation of CHO and B16F10 cells (Fig. 2B) treated with TK was significantly inhibited, whereas the proliferation of cells treated with mTK was unchanged. To confirm that the apoptosis was induced by TK, we labeled apoptotic cells with Annexin V-FITC and analyzed them by fluorescence-activated cell sorting and found that the amount of Annexin V–positive cells increased in response to TK in a time-dependent manner (Fig. 2C).

**TK-GFP Transduces into Tumor Cells In vivo**

To explore whether TK-GFP could transduce into tumor cells in vivo, TK-GFP or GFP was intratumorally administrated into B16F10 melanoma tumors implanted in syngeneic C57BL/6 mice. After 30 min, the tumors were excised, cryosectioned, and analyzed by fluorescence and confocal microscopy. TK-GFP was detected in the cytoplasm and nuclei of tumor cells (Fig. 3A, *c*); however, GFP was not
detected (Fig. 3A, b). 4′,6-Diamidino-2-phenylindole staining to identify cells was then conducted (Fig. 3A, a and d) and merged with TK-GFP and GFP (Fig. 3A, c and f). Serial z-sections of a single-cell image observed using confocal microscopy confirmed that TK-GFP transduced into tumor cells (Fig. 3B, 1-10).

**TK Administration Inhibits Tumor Growth**

To determine whether transduced TK could induce tumor cell apoptosis and inhibit tumor growth *in vivo*, we injected TK intratumorally daily for 8 days. As shown in Fig. 3C, administration of TK led to a striking reduction in tumor sizes compared with the size of tumors in mTK- or PBS-treated controls, and this difference was significant after the third day of treatment. As treatment continued, the differences between TK- and mTK- or PBS-treated groups became more pronounced until *P* < 0.001. To determine whether apoptosis was indeed the mechanism of tumor reduction *in vivo*, terminal deoxynucleotidyl transferase–mediated nick end labeling staining of the tumor sections was conducted. Terminal deoxynucleotidyl transferase–mediated nick end labeling staining revealed that treatment with TK induced apoptosis throughout ~70% of the total tumor volume, whereas mTK and PBS did not (Fig. 3D). No significant differences in body weight were observed between the groups (data not shown).

**Design and Characterization of TK3**

To design a more powerful and efficient antitumor peptide, we introduced more (KLAKLAK)2 peptides together with DEVD, a substrate peptide for caspase-3. TK1 and TK3 were named according to the numbers of (KLAKLAK)2 peptides that they contained. In some experiments, GFP was fused to each peptide, which were named TK1-GFP and TK3-GFP (Fig. 4A). To confirm that TK3 was cleaved by caspase-3, we incubated TK3-GFP with purified caspase-3. TK3-GFP was cleaved by caspase-3 in both dose- and time-dependent manners as indicated by the production of bands with lower molecular masses.

---

**Figure 6.** TK3 inhibits endothelial cell growth and angiogenesis. A, a, HUVEC were incubated with 1.5 μmol/L TK-GFP, mTK-GFP, or GFP for 2 h (×400); b, 81F10 melanomas grown in C57BL/6 mice were injected with 20 μmol/L TK-GFP. After 30 min, the tumors were cryosectioned and observed by microscopy. Arrows, microvessels in the tumor (×200). B, HUVEC were treated with concentrations of TK or mTK protein ranging from 0.5 to 15 μmol/L for 3 h at 37°C, and cell viability was then assessed using a MTT assay. Columns, mean of triplicate wells; bars, SE. C, HUVEC were seeded on Matrigel in the absence or presence of proteins (0.2 or 1 μmol/L) and then photographed after 3 to 5 h. The number of branch points was per five high-power field was counted for each sample. HPF, high-power field (×200). Columns, mean of triplicate wells; bars, SE. MTT assay and tube formation experiments were repeated three times. D, blood vessel quantification in TK3-treated tumors. Paraffin-embedded sections (3 μm) from tumor tissues were stained with an anti-CD31 antibody, and the number of CD31+ blood vessel was then counted. LPF, low-power field (×100). **, *P* < 0.01; ***, *P* < 0.001, versus PBS-treated control.
dramatic reduction of CD31+ blood vessels when compared with treatment with TK1 (Fig. 6D).

**Discussion**

A variety of approaches for the delivery of apoptosis-inducing peptides have been attempted (1–3); however, most of those approaches have focused on targeted delivery or efficient intracellular transduction. In this study, however, we designed a fusogenic peptide, TK3, using an approach that focused on transduction as well as potency, which was enhanced intracellularly by a caspase-3-mediated positive feedback loop. TK3 is composed of TAT followed by three repeats of (KLAKLAK)₂-DEV. Once TK3 transduces into cells, it activates caspase-3, which in turn cleaves DEVD to release more (KLAKLAK)₂ peptides. Therefore, one TK3 molecule releases three apoptosis-inducing peptides intracellularly. We used TAT for the intracellular delivery of the peptide because it is able to facilitate concentration-dependent delivery of fusion proteins into nearly all cells being treated (10, 17). We found that TK peptides rapidly transduce ~100% of cells in vitro and that TK uptake occurred in most of the cells in areas of the tumors that were injected with TK peptides, suggesting that TK peptides can transduce efficiently both in vitro and in vivo. TAT fusion proteins have an advantage at protein purification because it can be done regardless of denaturation. Misfolding of TAT fusion proteins could enhance their transduction into cells, where they are folded correctly and retain their bioactivities (17). All of our TK peptides that were purified under denaturation conditions appeared to be correctly folded in the inside cells because their apoptosis activities were not affected and their GFP forms were actively fluorescent in cells both in vitro and in vivo. The amphipathic peptide, (KLAKLAK)₂, does not disrupt the plasma membrane of eukaryotic cells. If internalized, however, it can disrupt the negatively charged mitochondrial membrane, resulting in apoptosis (18). Because deregulated cell proliferation of tumors coupled with suppressed apoptotic sensitivity after many treatments often renders tumors more progressive and resistant to any treatment, a strong and efficient initial treatment is required. Although (KLAKLAK)₂ has a powerful apoptosis-inducing activity, its antitumor effect is limited in vivo as shown in this article. Therefore, TK3, which was shown to have a stronger antitumor effect than TK1, was generated. TK3 induced cell growth inhibition at a 10-fold lower concentration than TK1 in vitro, and conversion of procaspase-3 to caspase-3 also occurred after administration of a significantly lower concentration of TK3 than TK1. This significant difference in potency does not seem to occur simply as a result of the difference in (KLAKLAK)₂ numbers at the same molar concentration, however, because the presence of three times the (KLAKLAK)₂ peptide numbers cannot account for >10-fold difference in activity. This difference does not appear to be due to a difference in their transduction efficiency either because both peptides use the same TAT domain, which transduces the protein in a dose-dependent manner, suggesting that a single transduced TK3 molecule more efficiently mediates apoptosis than three transduced TK1 molecules.
However, the precise mechanism by which this result occurs remains unclear at present. Interestingly, the *in vivo* effect of TK3 is more dramatic. We found that TK3-injected tumors were almost completely inhibited after 4 days of TK3 injection, whereas TK1-injected tumors were still growing, although their growth rates were much slower than those of PBS-injected tumors. TK3 also induced apoptosis in HUVEC, which suggests that TK3 can induce apoptosis of both tumor cells and vascular endothelial cells. Destroying both tumor cells and tumor vascular endothelial cells may result in a synergistic effect *in vivo*. In addition, a previous report (19) suggested that apoptosis plays a role in priming the immune response; therefore, TK3 may enhance the cellular immune response against tumor antigens by triggering massive apoptosis in the tumor milieu.

Although intratumoral injection of TK3 shows an excellent antitumor activity *in vivo*, we need to develop a way to deliver TK3 systemically. TK3 can be linked to a tumor-specific target moiety such as RGD sequence that has been successfully used for the delivery of a short peptide (20, 21). It can be also delivered to the tumor site by “passive tumor targeting” with the help of particles that can carry TK3 by making physical complex or by chemical conjugation (22–25). Taken together, our data show that a peptide that amplifies the caspase-3 activation signal by transducing tandem repeats of (KLAKLAK)$_2$-DEVD sequences into cells exhibited potent antitumor activities both *in vitro* and *in vivo*. Therefore, this study serves as the prototype for a peptide-based drug with the potential to reduce tumor burden when used alone or in combination with conventional therapies.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

**References**


15. Nam JC, Jeong HW, Lee BH, Park RW, Kim IS. Regulation of tumor angiogenesis by fastatin, the fourth FAS1 domain of *igh*3-h, via $\alpha h3\beta2$ integrin. Cancer Res 2005;65:4153–61.


Molecular Cancer Therapeutics

Antitumor effect of a transducible fusogenic peptide releasing multiple proapoptotic peptides by caspase-3

Mi-Kyung Kwon, Ju-Ock Nam, Rang-Woon Park, et al.


**Updated version**
Access the most recent version of this article at:
http://mct.aacrjournals.org/content/7/6/1514

**Cited articles**
This article cites 25 articles, 8 of which you can access for free at:
http://mct.aacrjournals.org/content/7/6/1514.full.html#ref-list-1

**Citing articles**
This article has been cited by 1 HighWire-hosted articles. Access the articles at:
/content/7/6/1514.full.html#related-urls

**E-mail alerts**
Sign up to receive free email-alerts related to this article or journal.

**Reprints and Subscriptions**
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

**Permissions**
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.