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利用癌症專一性啟動子 Survivin 結合細胞凋亡基因 BIK 進行非小細胞肺癌之基因治療。

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Cancer-Specific Activation of the Survivin Promoter and Its Potential Use in Gene Therapy[1]

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ABSTRACT

Survivin is expressed in many cancers but not in normal adult tissues and is transcriptionally regulated. To test the feasibility of using the survivin promoter to induce cancer-specific transgene expression in lung cancer gene therapy, a vector expressing a luciferase gene driven by the survivin promoter was constructed and evaluated *in vitro* and *in vivo*. We found that the survivin promoter was generally more highly activated in cancer cell lines than in normal and immortalized normal cell lines. When delivered intravenously by DNA:liposome complexes, the survivin promoter was more than 200 times more cancer specific than the cytomegalovirus promoter *in vivo*. To identify lung cancer patients who may benefit from gene therapy with the survivin promoter, we measured survivin protein expression in surgical specimens of 75 non-small-cell lung cancers and 10 normal lung tissues by immunohistochemical staining and found that survivin is expressed in most of the non-small-cell lung cancers tested (81%, 61 of 75) but none of the normal lung tissues. The survivin promoter also induced transgene expression of a mutant Bik in cancer cells, which suppressed the growth of cancer cells *in vitro* and *in vivo*. These results indicate that the survivin promoter is a cancer-specific promoter for various cancers and that it may be useful in cancer gene therapy.

INTRODUCTION

Lung cancer is one of the leading causes of death worldwide.[1] Survival has not substantially increased recently, although vigorous efforts have been made to improve it. As the pathogenesis and biology of tumors are becoming clear, gene therapy could be an effective alternative for the treatment of lung cancer. Non-viral gene delivery systems such as cationic lipids are attractive strategies for therapeutic application because of their minimal immunogenicity and low toxicity *in vivo*. (N-[1-(2,3-Dioleoyloxy)]-N,N,N-trimethylammonium propane methylsulfate (DOTAP): cholesterol (Chol) cationic liposomes have been reported to be good preparations for mediating efficient gene transfer to various organs, especially to the lungs.[2,3] One study showed that therapeutic tumor suppressor genes delivered by this improved liposome vector can inhibit primary and disseminated human non-small-cell lung cancers in animal models.[3]

Most gene therapy approaches currently use nonspecific, nonselective prokaryotic promoters such as the cytomegalovirus (CMV) promoter that can be expressed at high levels in normal cells, potentially contributing to toxicity. This is particularly true when cytotoxic or strongly apoptotic genes are used. For cancer-specific expression of cytotoxic or apoptotic genes, a better approach would be to use a tissue- or cancer-specific promoter to limit the spectrum of cells that express the gene therapy construct.[4, 5]

Possible for such therapy, survivin is a member of the IAP (inhibitor of apoptosis) family. Survivin is overexpressed in common cancers but not in normal adult tissues.[6-8] Survivin mRNA or protein overexpression (compared with normal tissues) was demonstrated in tumors of the lung,[9, 10] breast,[11] colon,[12] stomach,[13] esophagus,[14] pancreas,[15] bladder,[16] uterus,[17] ovaries[18] and skin,[19] and in lymphoma[20] and

leukemia.[21] Transcription experiments indicated that survivin protein expression in cancer tissue appears to be regulated, at least in part, transcriptionally.[22, 23] In a genome-wide search, survivin constituted the fourth top "transcriptome" in cancers of lung, colon, and brain and melanoma, but was weakly expressed or undetectable in the same normal tissues.[24] Analyses of survivin mRNA expression levels in non-small-cell lung cancers (NSCLC) also revealed a strong survivin mRNA up-regulation in nearly all tumor samples compared with non-tumor controls.[10, 25] Because of the cancer-specific expression of survivin, some groups have developed antisense RNA[26] or dominant negative mutant protein[27] for cancer therapy.[28] However, cancer-selective activation of the survivin promoter *in vivo* has not been demonstrated, and the survivin promoter has not been used in cancer gene therapy.

Bik is a proapoptotic member of the Bcl-2 gene family, which triggers apoptosis through a p53-independent pathway.[29, 30] Previously, we have shown the effectiveness of tumor suppression by systemic delivery of Bik:liposome complexes.[31] We have also demonstrated that a mutant form of Bik (BikDD) is more potent than the wild type Bik in killing cancer cells.[32]

Because survivin is generally strongly expressed in cancer cells but repressed in most normal cells and because its expression is regulated at the transcriptional level, we tested whether the survivin promoter can be used in lung cancer gene therapy. We examined the specificity of survivin promoter activation in lung cancers and the prevalence of survivin expression in lung cancer specimens. We also evaluated the tumor-inhibitory effect of the proapoptotic gene BikDD under the control of the survivin promoter.

MATERIALS AND METHODS

Liposomes and Cell Lines

DOTAP:Chol stock solution (20mM) was prepared by the Non-Viral Core facility at the Baylor College of Medicine (Houston, TX) directed by Nancy Smyth Templeton. The human cancer cell lines H1437, H358, H1435, H322, and H1299 (non-small-cell lung cancer); Hela (cervical cancer); and HT29 (colon cancer) and the normal and immortalized normal cell lines WI-38 (normal lung fibroblast) and 184A1 (normal mammary epithelial cells, chemically immortalized) were purchased from the American Type Culture Collection (Manassas, VA) and cultured according to the vendor's instruction.

Construction of Plasmids

Four different plasmids were constructed (Fig. 1). To make the luciferase expression plasmid under the control of the survivin promoter (pSRVN-Luc), we generated a 977-base-pair fragment of the human survivin gene promoter (nucleotides 1824-2800, GenBank accession number U75285) by polymerase chain reaction of human bacterial artificial chromosome libraries (RPC 1-11, 0219G17, ResGen, Inc., CA) as a template. The sequences of the oligonucleotides primers were as follows: forward primer, 5' ATA CGA GAT CTG CCA TAG AAC CA 3' and reverse primer, 5' ATG TAA AGC TTC CAC CTC TGC CA 3'. After restriction enzymes digestion and purification, the fragment was inserted into the luciferase vector pGL3-basic (Promega, Madison, WI) at the *Bgl*II and *Hind*III sites. To make a luciferase expression plasmid under the control of the CMV promoter (pCMV-Luc), we excised the 654-base-pair CMV promoter from the vector pcDNA3 (Invitrogen, San Diego, CA) by digestion with *Bgl*II and *Hind*III and inserted it into pGL3-basic. To make the

BikDD constructs, pSRVN-BikDD and pCMV-BikDD, we replaced the luciferase gene of pSRVN-Luc and pCMV-Luc with BikDD by *Hind*III and *Xba*I digestion. For an empty experimental control plasmid pGL3-basic-CD, we used a promoterless pGL3-basic vector in which the luciferase gene was replaced with the harmless cytosine deaminase gene. These plasmids were confirmed by sequence analysis.

Transient Transfection and Reporter Assays

To normalize transfection efficiency, we used the Dual-Luciferase reporter assay system according to the manufacturer's instructions (Promega). The dual luciferase assay is a widely used technique[33, 34]. In this system, the plasmid pRL-TK which contains the *Renilla* luciferase gene under the control of the thymidine kinase promoter is co-transfected as an internal control. In our previous study[33] and preliminary tests, the survivin and CMV promoters have no obvious effects on the transgene expression of the thymidine kinase promoter. Cells were grown in 6-well plates with the suggested medium until 60-80% confluent. Then, the cells were transiently transfected with 1.5 μg of tested plasmid DNA along with 0.1 ug of pRL-TK using 1.6 ul of liposomes (DOTAP:Chol, 8mM) in 200 ul of Opti-Medium (Life Technology) per well for 4-6 h. Cell lysates were prepared 48 h after transfection for the dual luciferase assay by following the manufacturer's instructions (Promega) and using a TD-20/20 Luminometer (Turner Designs, Sunnyvale, CA). The dual luciferase ratio was defined as the luciferase activity of the tested plasmids divided by the luciferase activity of pRL-TK.

Preparation of DNA:Liposome Mixtures for Animal Studies

DNA:liposome complexes were prepared fresh 2 to 3 h before tail vein or

intratumoral injection into mice as previously described.[2] Briefly, DOTAP:Chol (20 mM) stock solution and stock DNA solution were diluted in 5% dextrose in water (D5W) and mixed in equal volumes to make a final solution of 4 mM DOTAP:Chol and 150 μg DNA in 300 μl of solution (liposome:DNA ratio 1:2.6). All reagents were diluted and mixed at room temperature in a 1.5-ml Eppendorf tube. The DNA solution was added at the surface of the liposome and mixed rapidly up and down twice with the pipet tip. The DNA:liposome mixture was precipitate free and used for all *in vivo* experiments.

Establishment of Tumors

Before the start of the animal experiments, nu/nu mice (18-22 g; Charles River Laboratories, Wilmington, MA) were subjected to 3.5 Gy of total body irradiation from a cesium source to increase tumor uptake. All of the mice were cared for according to the Guide for the Care and Use of Laboratory Animals (NIH publication number 85-23) and the institutional guidelines of The University of Texas M. D. Anderson Cancer Center. Then, 5 x 10^6 H1299 cells were inoculated subcutaneously into the right dorsal flanks of 6- to 8-week old nu/nu mice to establish tumors.

Tissue Distribution of the Liposome-Delivered Reporter Genes

H1299 cells were subcutaneously inoculated into the right dorsal flank of the nude mice. Fourteen days later until the tumor size reached 6-8 mm, the tumor-bearing mice received a single injection of 100 μl of DNA:liposome complexes containing 50 μg of DNA through the tail vein using a 29-gauge syringe needle. Forty-eight hours after injection, the mice were killed, and the tumors and other organs were resected and immediately froze on dry ice. The tissues were homogenized after adding 1X

passive lysis buffer (Promega Corp., Madison, WI) with a volume (μl) equivalent to ten times the tissue weight (mg). After undergoing a freeze-thaw procedure, the tissue suspension was centrifuged at 12,000 rpm, 4° C, for 3 min and the supernatant was collected for measurement of luciferase activity and protein concentration. The luciferase activity in the tissue supernatant was measured using a Lumat LB9507 instrument (Berthod, Bad Wildbad Germany). The luciferase activity (relative light unit, RLU) per mg protein was used to compare gene expression in different tissues. The background levels of luciferase activity in tumor and organs were measured using 3 tumor-bearing mice with intravenous injection of empty plasmid-liposome complexes. Protein concentrations were determined by using Bio-Rad protein assay reagent (Bio-Rad, Fremont, CA).

Tumor Samples and Immunohistochemical Staining

Archived surgical specimens from 75 non-small-cell lung cancers (46 adenocarcinomas, 24 squamous cell carcinomas, and 5 adenosquamous carcinomas) and 10 normal lung tissues (obtained at least 5 cm from the tumor or from a different lobe of the involved lung) were analyzed in this study. Expression of survivin was evaluated by immunohistochemical staining. Briefly, after deparaffinization and antigen retrieval, 5 μm tissue sections were incubated overnight at 4°C with polyclonal anti-survivin antibody (diluted 1:200, NOVUS Biologicals Inc., Littleton, CO). The sections were then incubated with biotinylated goat antirabbit immunoglobulin G (Vector Laboratories, Burlingame, CA) and subsequently incubated with avidin-biotin-peroxidase complex (Vector Laboratories). Color development was performed with the 0.125% aminoethylcarbazole chromogen substrate solution (Sigma-Aldrich, Union City, CA). For a negative control, all

incubation steps were identical except that phosphate-buffered saline rather than primary antibody was used. A previously identified strongly staining tumor tissue section was used as a positive control. The mean percentages of positive tumor cells were determined in at least five areas at 100X or 200X magnification. Tissues were scored positive when more than 10% of the cells reacted with anti-survivin antibody. The results were analyzed and confirmed by two individuals.

Growth Inhibition of Human Cancer Cells *In Vitro*

A transient transfection assay was used to evaluate the cell growth-inhibiting effect of pSRVN-BikDD. In brief, cells were grown in 6-well plates with the suggested medium until 60-80% confluent. A fixed amount (50 ng) of pCMV-Luc was co-transfected with 0, 1.5, or 3 μg of pSRVN-BikDD into various human cancer cells per well for 4-6 h. The final DNA and lipid amounts in each well were kept constant (3.05μg DNA and 3.05 ul of 8mM DOTAP:Chol, respectively) by adding an appropriate amount of the pGL3-basic-CD vector. The cells were harvest 48 h after transfection, and cell lysates were prepared for the luciferase assay. Because luciferase activity is indicative of living cells, the relative luciferase activity was used as an index of cell growth and proliferation[32]. For direct measurement of cell viability, live cells of each treatment were counted manually using Trypan Blue staining (Gibco-BRL) and hemacytometer. For apoptosis assay, Annexin V detection kit (BD Biosciences) was used following the manufactory's instructions. For western blot analysis of BikDD expression, cells were harvest 24 h after transfection.

Tumor Growth and Treatments *In Vivo*

H1299 cells were subcutaneously inoculated into the right dorsal flank of 14

nude mice. When the tumor had reached 4-5 mm^2 (8 days after inoculation), the animals were randomized into 2 groups (7 mice in each group), and intratumoral treatment was initiated. Group 1 received DOTAP:Chol-pCMV-Luc complex (vector control) and group 2 received DOTAP:Chol-pSRVN-BikDD complex (50 μg/dose for each group). Treatments were given every other day for a total of six doses. The tumors were measured every other day, and the tumor volumes were calculated by using the formula V (mm^3) = a x $b^2/2$, where a is the largest dimension and b is the perpendicular diameter. Antitumor efficacy data are presented as cumulative tumor volumes for all animals in each group. The statistical significance of the experimental results was calculated by using Student's *t* test for tumor measurements.

RESULTS

Cancer-Specific Transgene Expression of the Survivin Promoter *In Vitro*

To assess whether the activation of the survivin promoter is cancer-specific and to compare the cancer-specificity between the survivin promoter and the commonly used CMV promoter, we used pSRVN-Luc and pCMV-Luc to determine the activity of the survivin and CMV promoters in cancer and normal cell lines. As shown in figure 2, the survivin promoter activity was generally very high in cancer cell lines while very low in normal cell lines. When compared with the activity of the CMV promoter, although the survivin promoter was weaker, the survivin promoter demonstrated greater transgene expression difference between the cancer and normal cell lines, indicating that the survivin promoter is more specifically activated in cancer cells than in normal cells and that the cancer-specificity of transgene expression of the survivin

promoter was better than the CMV promoter.

Greater Cancer Specificity of the Survivin Promoter than the CMV Promoter *In Vivo*

To determine how cancer specific the transgene expression induced by the survivin promoter *in vivo* is, we established subcutaneous H1299 tumors in nude mice and infused 50 μg/100 μl of pSRVN-Luc or pCMV-Luc into each mouse via the tail vein. The tumors, hearts, lungs, livers, spleens, kidneys, and muscle were removed 48 hrs later for luciferase assay. High levels of luciferase activity were detected in the lungs and hearts of the mice treated with pCMV-Luc (Fig. 3A). When compared with the luciferase activity in the tumor, the activity was 42- and 18-fold higher in the lung and heart, respectively. In contrast, the luciferase activity in all the normal tissues of mice treated with pSRVN-Luc was lower than that in tumors (Fig. 3B), and the activity was only 0.19 and 0.48 fold in the lung and heart, respectively. For systemic gene therapy in lung cancers, the expression of therapeutic genes must be high in tumors to increase the genes' anti-tumor effects, and the expression in normal lung tissue should be low to decrease potential toxicity. Therefore, we defined the cancer-specific index as the luciferase activity of tumors divided by the activity of the lungs. The cancer-specific index was 0.02 for pCMV-Luc, and 5.13 for pSRVN-Luc, indicating that the survivin promoter was more than 200 times more cancer specific than the CMV promoter.

Survivin Is Expressed in Most Human Non-Small-Cell Lung Cancers but Not in Normal Lung Tissues

To determine the potential population of lung cancer patients who may benefit from gene therapy with the survivin promoter, the prevalence and expression status of

survivin in cancer and normal lung tissues were determined using archived surgical specimens from 75 non-small-cell lung cancer patients and 10 normal lung tissues by immunohistochemical staining. Representative pictures of cancer and normal lung tissues are shown in Fig. 4. Survivin staining was mainly located in the cytoplasm. Occasional nuclear staining was also observed. Survivin was expressed in 61 out of 75 (81%) non-small-cell lung cancer specimens but in none of the 10 of normal lung tissues. The prevalence of survivin expression in different subtypes of lung cancers was similar, 80% (37 of 46) in adenocarcinomas, 83% (20 of 24) in squamous cell carcinomas, and 80% (4 of 5) in adenosquamous carcinomas. These results not only supported the finding that activation of the survivin promoter is cancer specific but also suggested that gene therapy with the survivin promoter can be applied to many lung cancer patients.

Expression of BikDD Driven by the Survivin Promoter Inhibits the Cancer Cells Growth *In Vitro* and *In Vivo*

To evaluate the possibility of using the survivin promoter for *in vitro* gene therapy, a BikDD expression plasmid containing the survivin promoter (pSRVN-BikDD) was constructed. We performed a transient co-transfection assay to determine the cell growth inhibitory effect of pSRVN-BikDD. As shown in Fig. 5A, when compared with the luciferase activity of cells without pSRVN-BikDD treatment (0 μg/well), the luciferase activity of three cancer cell lines decreased after treatment of pSRVN-BikDD in a dose dependent manner. Western blot analysis showed that BikDD was expressed after pSRVN-BikDD transfection (Fig. 5B). To directly measure the growth inhibitory effect of pSRVN-BikDD, vital cells were counted by Trypan Blue staining and showed that pSRVN-BikDD treatment (3 μg/well) killed

24-30% cells depending on the cell line we used (Fig. 5C). The cell killing efficiencies are consistent to the transfection efficiencies that are around 30% (data not shown, examined by green fluorescence protein DNA transfection). Annexin-V detection also showed that the pSRVN-BIKDD treatment (3 μg/well) induced apoptosis of cancer cells, although the efficacy was less when compared with pCMV-BIKDD (a 14% killing effect compared to pCMV-BikDD, which induced 30-80% apoptosis of cancer cells[32], Fig. 5D). These data consistently indicated that the survivin promoter can drive BikDD expression, and induce apoptosis and inhibition of growth in multiple types of cancer cell lines.

To evaluate the possibility of using the survivin promoter for *in vivo* gene therapy, we established subcutaneous H1299 tumors in nude mice and treated the tumors with pSRVN-BikDD and pCMV-BikDD. After six sequential intratumoral injections of DNA:liposome complexes, the treatment groups of pSRVN-BikDD and pCMV-BIKDD inhibited tumor growth statistically significantly more than did treatment with pCMV-Luc (Fig. 5E). These results demonstrated that the survivin promoter effectively drives BikDD expression in cancer cells and so inhibits the growth of cancer cells *in vitro* and *in vivo*.

DISCUSSION

Advanced lung cancer patients usually have compromised pulmonary function because of a long history of smoking or tumor-associated respiratory insufficiency. Targeted expression of therapeutic genes with reduced toxicity of major organs, especially in the lungs, is critical for systemic gene therapy of these patients. Among

the numerous targeted gene strategies, control of gene expression via tissue- or cancer-specific promoters is an attractive strategy and has been tested extensively. By using non-specific prokaryotic promoters such as the CMV promoter delivered by cationic liposomes, the levels of transgene expression in normal lung tissues could be as high as 100-fold compared with distant tumors.[35] Our data also revealed that the ratio of the CMV promoter activity in the lung and heart was 42- and 18-fold higher than in the tumors, respectively. A previous report also demonstrated the high gene expression in the alveolar epithelial cells (type II pneumocytes) and endothelial cells of the lungs after tail vein injection of DOTAP:Chol liposome complexed with CMV-LacZ plasmid DNA.[3] These data consistently demonstrated that the CMV promoter is highly activated in normal lung tissues and may contribute to pulmonary toxicity when used in systemic gene therapy.

The expression of survivin is noted in common human cancers but not in normal adult tissues. Because the increased survivin activity is controlled transcriptionally, it has been suggested that the survivin promoter might control the transgene expression in a cancer-specific manner.[23] However, this hypothesis has never been proved *in vivo*. In the study reported here, we demonstrated that the survivin promoter was strongly activated in various cancer cell lines but repressed in normal cell lines and tissues, especially in the lungs. In fact, the activity of the survivin promoter expression in the normal tissues of the heart, lung, liver and kidney was very low, with almost no expression when compared with the background. These results suggest that when a systemically administered suicide gene is driven by the survivin promoter, the systemic toxicity could be significantly reduced.

Most cancer-specific promoters are only activated in a limited population of tumor histologies. To determine whether the survivin promoter can be applied universally in

gene therapy of lung cancer patients, survivin protein expression was evaluated by immunohistochemical staining in surgical specimens of 75 NSCLC patients. Consistent with two previous publications that demonstrated that over than 95% NSCLC samples examined strongly overexpressed survivin mRNA and protein compared with a non-tumor control[10, 25], and that the survivin protein was overexpression in most of the NSCLC specimens (81 %) regardless of histological subtypes. In addition, the general activation of the survivin promoter and similar inhibitory effects of the pSRVN-BikDD in lung, colon, and cervical cancer cell lines also suggested that using the survivin promoter in gene therapy may benefit patients with many kinds of cancer. Furthermore, the depressed expression of survivin in all normal lung tissues also supports our hypothesis that the activation of the survivin promoter is cancer-specific and, when used in gene therapy, could reduce the pulmonary toxicity.

Another limitation of cancer-specific promoters is that most of these promoters are much weaker than commonly used viral promoters such as the CMV promoter or the SV40 promoter.[36] Although the activity of the survivin promoter was about 10 to 40 % of the CMV promoter in all cancer cells tested, the transgene expression of the survivin promoter was 2-to 10-fold higher than the SV40 promoter (data not shown). A previous report also showed that the survivin promoter is 2- to 3-fold more active than the SV40 promoter,[23] suggesting that the survivin promoter can induce high transgene expression in cancer cells.

In summary, our study demonstrated that the activation of the survivin promoter is cancer specific and can effectively induce transgene expression in cancer cell lines and xenograft tumors. The survivin promoter can also drive expression of BikDD in cancer cells inhibiting cancer cell growth *in vitro* and *in vivo*. Because survivin

expression is commonly expressed in lung cancer specimens and various cancer cell lines, the survivin promoter may be useful in cancer gene therapy.

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LEGENDS

Fig. 1. Plasmid constructs. In the luciferase reporter plasmids, the promoters were cloned upstream of the luciferase gene in the restriction sites shown. pSRVN-BikDD and pCMV-BikDD were constructed by replacing the luciferase gene with the BikDD gene. Luc, luciferase gene.

Fig. 2. *In vitro* analyses of transgene expression of the survivin and CMV promoters by transient transfection. The transcriptional activity of the survivin and CMV promoters was measured in various cancer, normal (WI-38), and immortalized normal (184A1) cells. The data shown are the means of at least three independent experiments; the error bars indicate the standard deviation (SD).

Fig. 3. *In vivo* analyses of transgene expression of the CMV and survivin promoters by intravenous delivery of DNA:liposome complexes into tumor-bearing mice. The luciferase activity of tumor and major organs were measured at 48h after treatment of (A) pCMV-Luc, and (B) pSRVN-Luc. The error bars indicate the standard deviation; n = 5 in each group. Luciferase activity is expressed as relative light unit (RLU)/mg protein and luciferase gackground levels (100-200 RLU/mg protein) were subtracted from each measurement.

Fig. 4. Representative immunohistochemical staining for survivin expression in A, lung cancers and B, normal lung tissues. The arrows indicate non-specific immunostaining of survivin in macrophages. (Original magnification, 200X)

Fig. 5. Inhibition of growth of cancer cells by pSRVN-BikDD *in vitro* and *in vivo*. A, H1299, Hela, and HT-29 cells were co-transfected with 50 ng of pCMV-luc and increasing amount (0, 1.5, or 3 μg/well) of pSRVN-BikDD. The relative luciferase activities were calculated by setting the luciferase activities obtained from transfections without pSRVN-BikDD (0 μg/well) at 100%. The data are the means of at least three independent experiments; the error bars indicate the SD. B, induction of BikDD gene expression in H1299 cells after treatment with optimal medium only (lane 1), pGL3-basic-CD vector (3μg/well)-liposome complexes (lane 2), and pSRVN-BikDD (3μg/well)-liposome complexes (lane 3). Western blot was performed 24 h after treatments. C, H1299, Hela, and HT-29 cells were transfected with 3 μg of pSRVN-Luc (control) or pSRVN-BikDD. The vital cell numbers were counted using Trypan Blue exclusion method after 24 hours. D, H1299 cells were transfected with either 3 μg of pSRVN-Luc, pSRVN-BikDD or pCMV-BikDD. The apoptotic cell numbers were counted using Annexin V detection method after 24 hours. The relative apoptosis ratio was calculated by setting the apoptotic cell numbers by pSRVN-Luc treatment as 0% and the apoptotic cell numbers by pCMV-BikDD treatment at 100%. E, Suppression of tumor growth by pSRVN-BikDD and pCMV-BikDD. Tumor volume was monitored every other day after inoculation of tumor cells. The arrows indicate the time points at which treatment was given. The vector control was pCMV-Luc. $^{*}p < 0.01$ when compared with pSRVN-BikDD or pCMV-BikDD. The tumor suppression effects between pSRVN-BikDD and pCMV-BikDD were not significantly different, n = 7 in each group.

Fig. 1

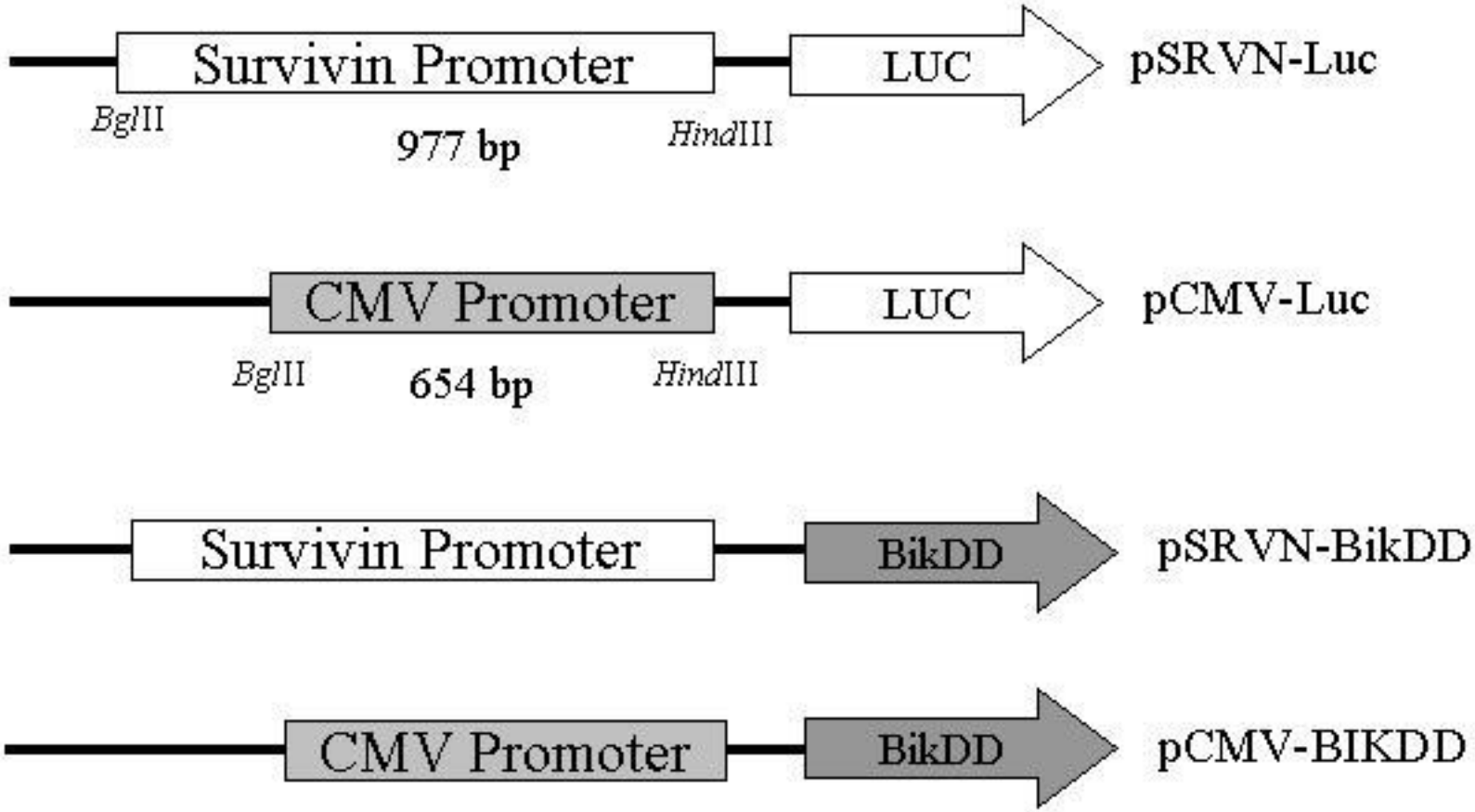

Survivin Promoter
LUC
pSRVN-Luc
BglII
977 bp
HindIII
CMV Promoter
LUC
pCMV-Luc
BglII
654 bp
HindIII
Survivin Promoter
BikDD
pSRVN-BikDD
CMV Promoter
BikDD
pCMV-BIKDD

Fig. 2

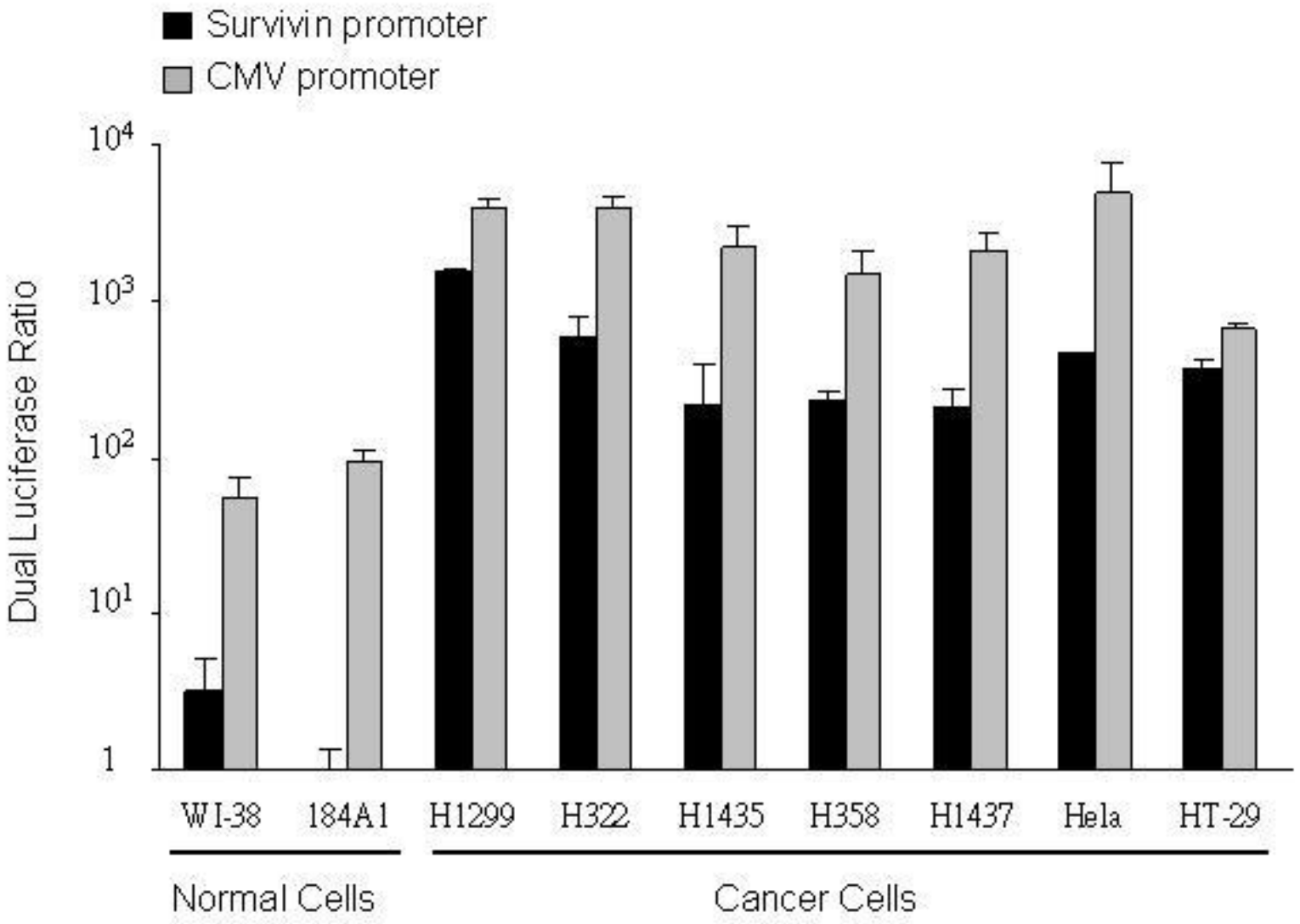

Survivin promoter
CMV promoter
Dual Luciferase Ratio
10^4
10^3
10^2
10^1
1
WI-38
184A1
H1299
H322
H1435
H358
H1437
Hela
HT-29
Normal Cells
Cancer Cells

Fig. 3

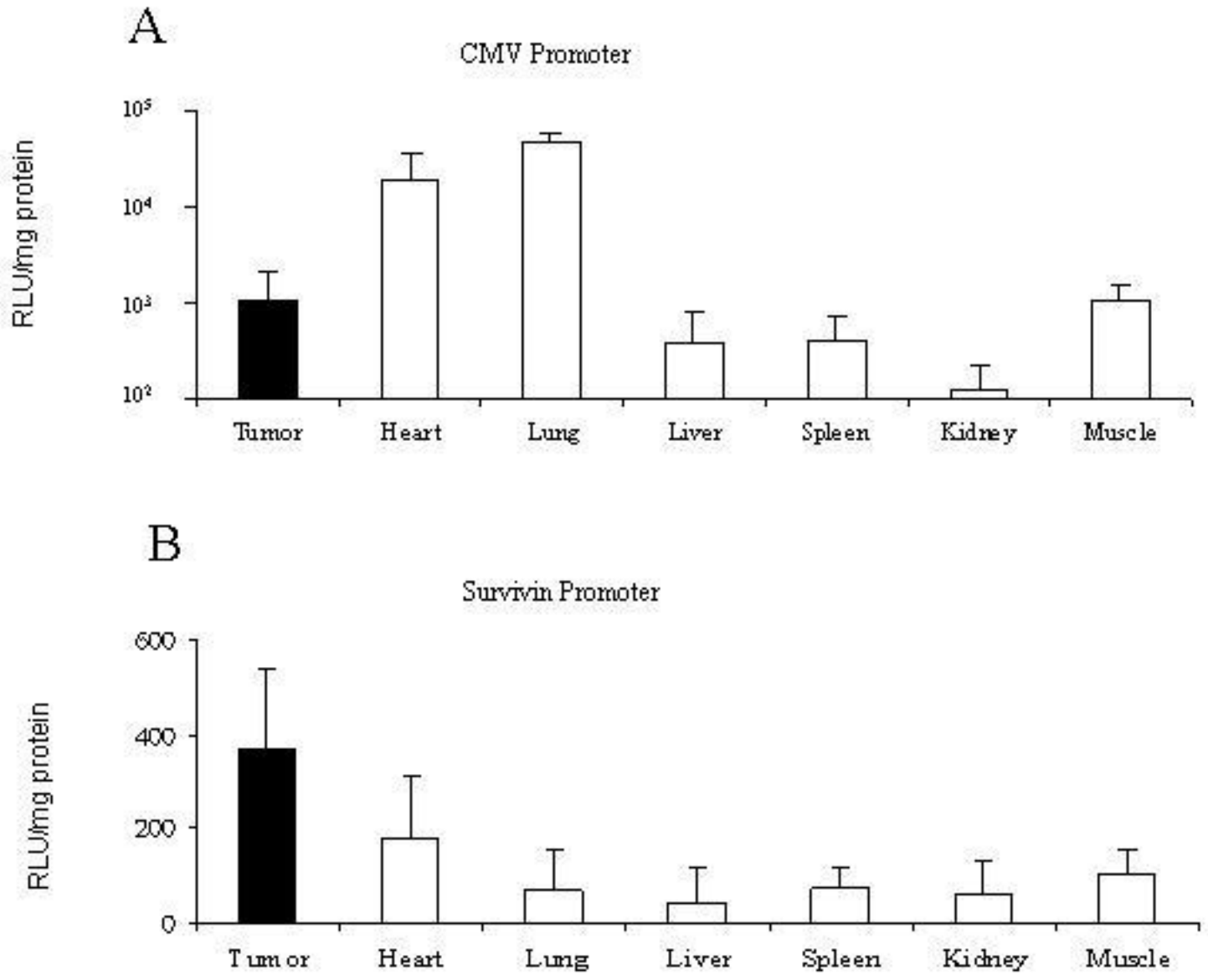

A
CMV Promoter
RLU/mg protein
10^5
10^4
10^3
10^2
Tumor
Heart
Lung
Liver
Spleen
Kidney
Muscle
B
Survivin Promoter
RLU/mg protein
600
400
200
0
Tumor
Heart
Lung
Liver
Spleen
Kidney
Muscle

Fig. 4

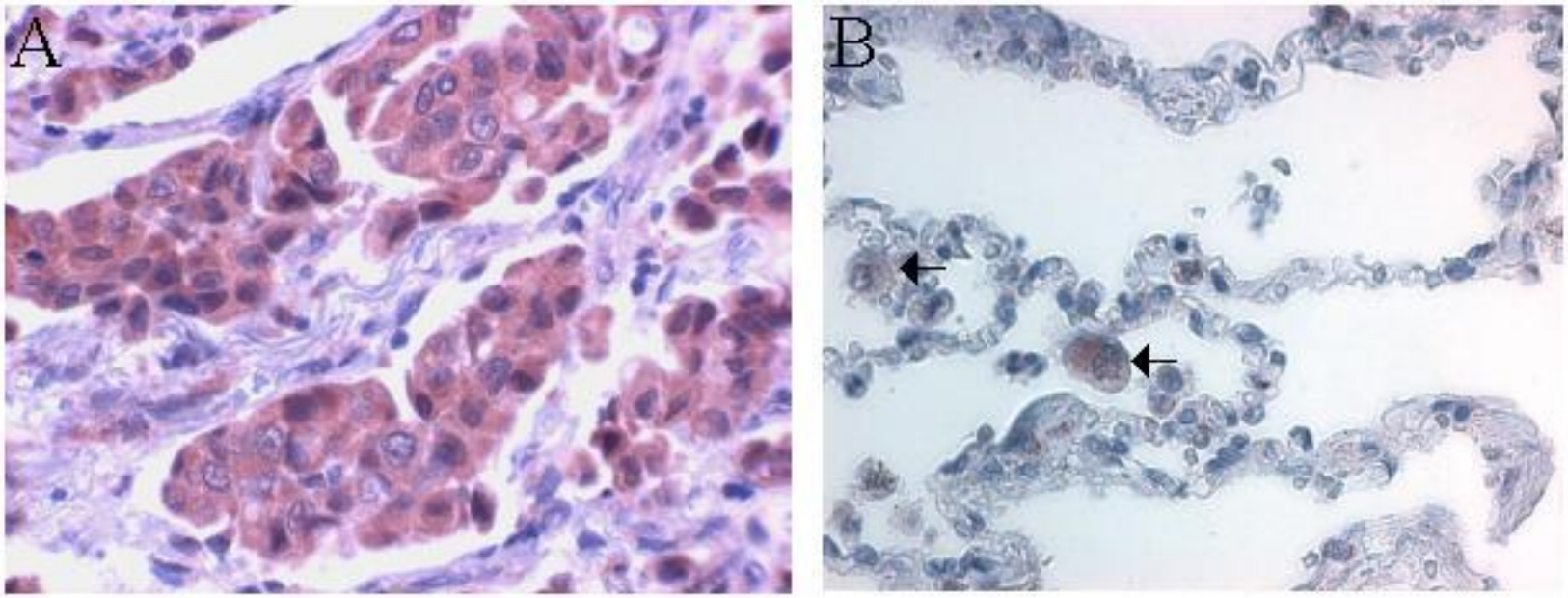


Fig. 5A

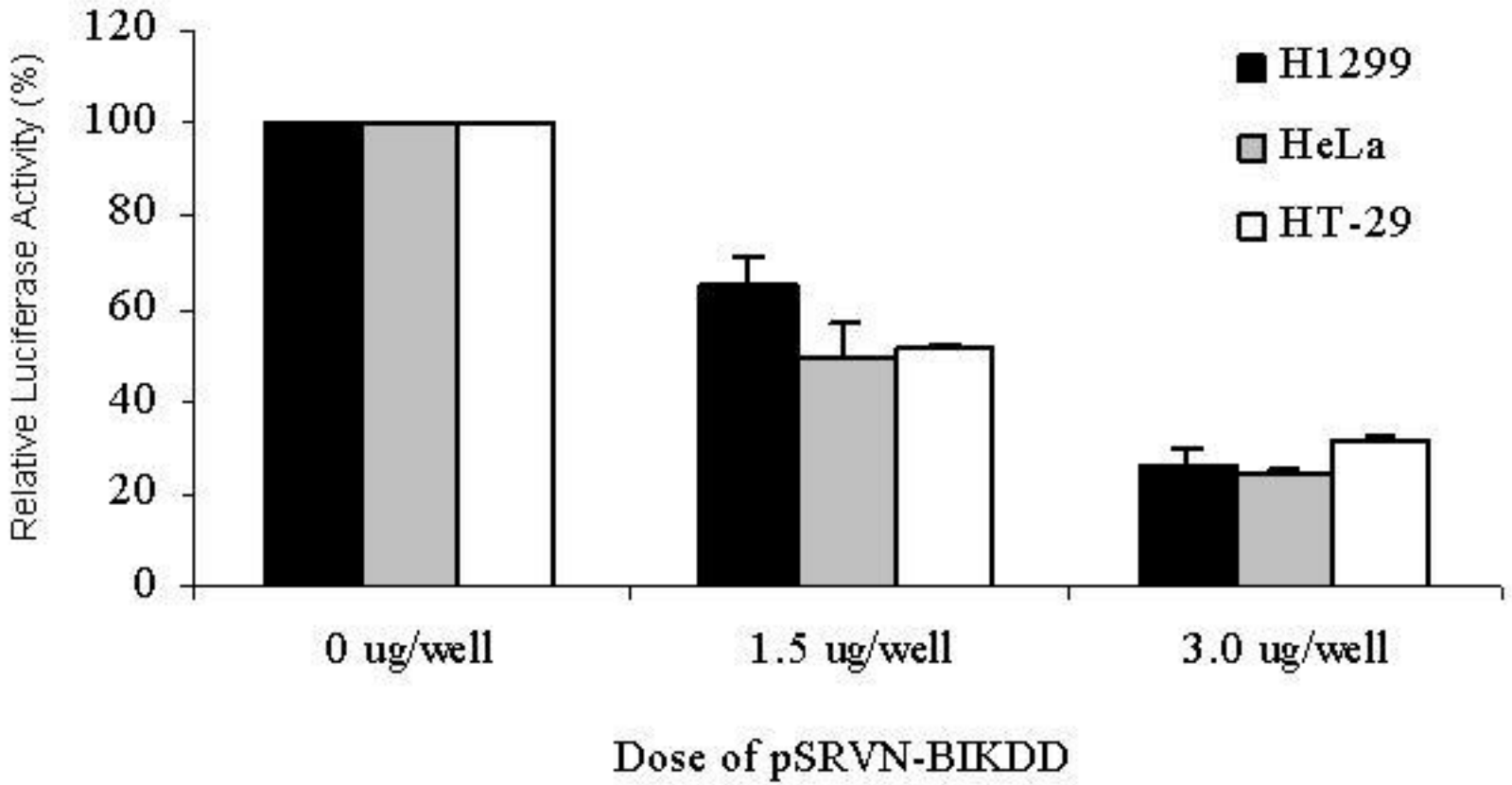

120
100
80
60
40
20
0
Relative Luciferase Activity (%)
H1299
HeLa
HT-29
0 ug/well
1.5 ug/well
3.0 ug/well
Dose of pSRVN-BIKDD

Fig. 5B

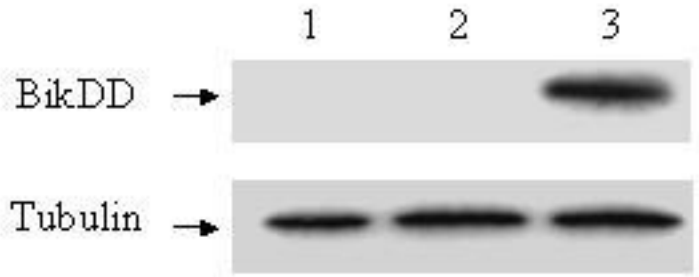


Fig. 5C

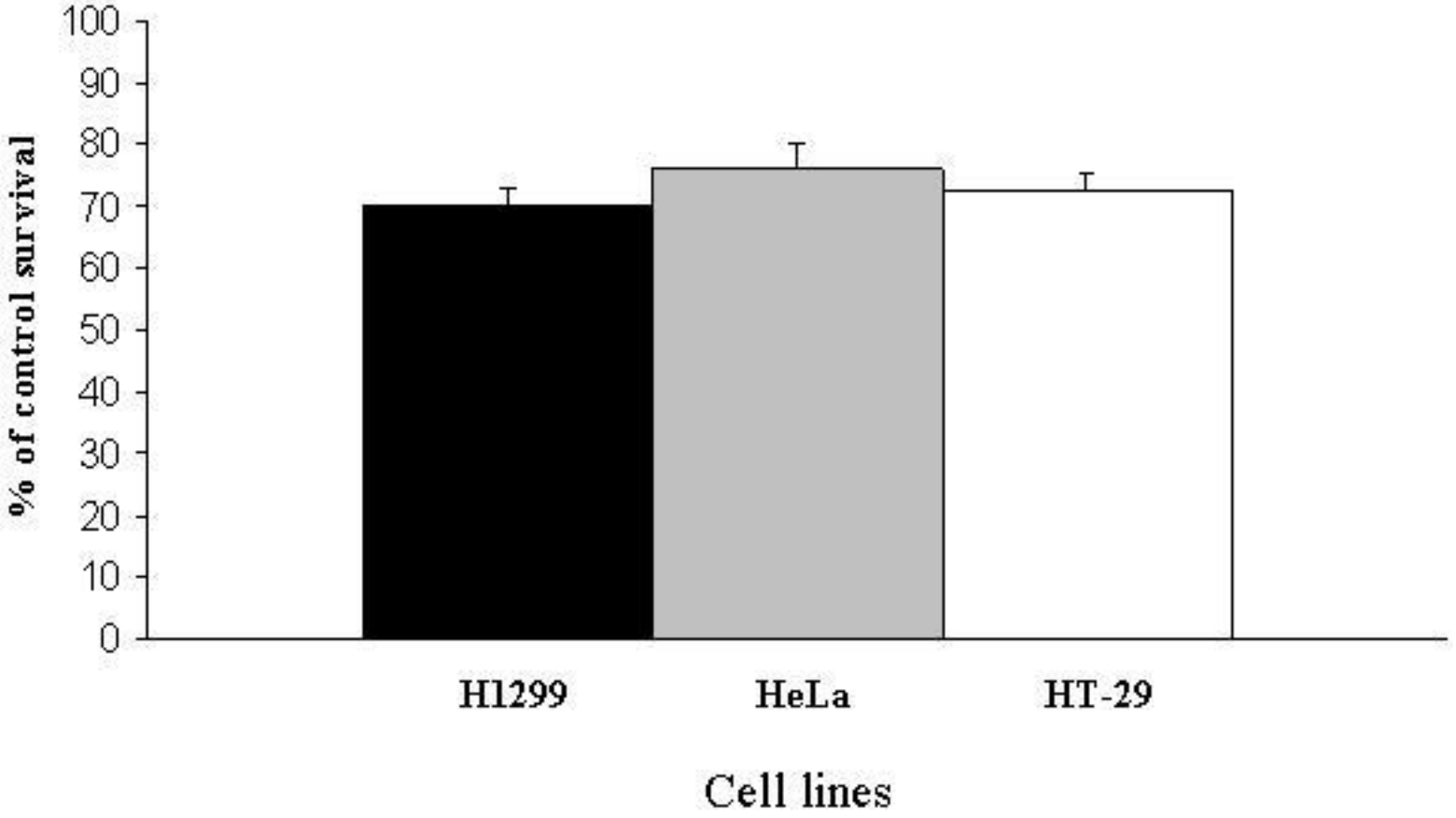

100
90
80
70
60
50
40
30
20
10
0
% of control survival
H1299
HeLa
HT-29
Cell lines

Fig. 5D

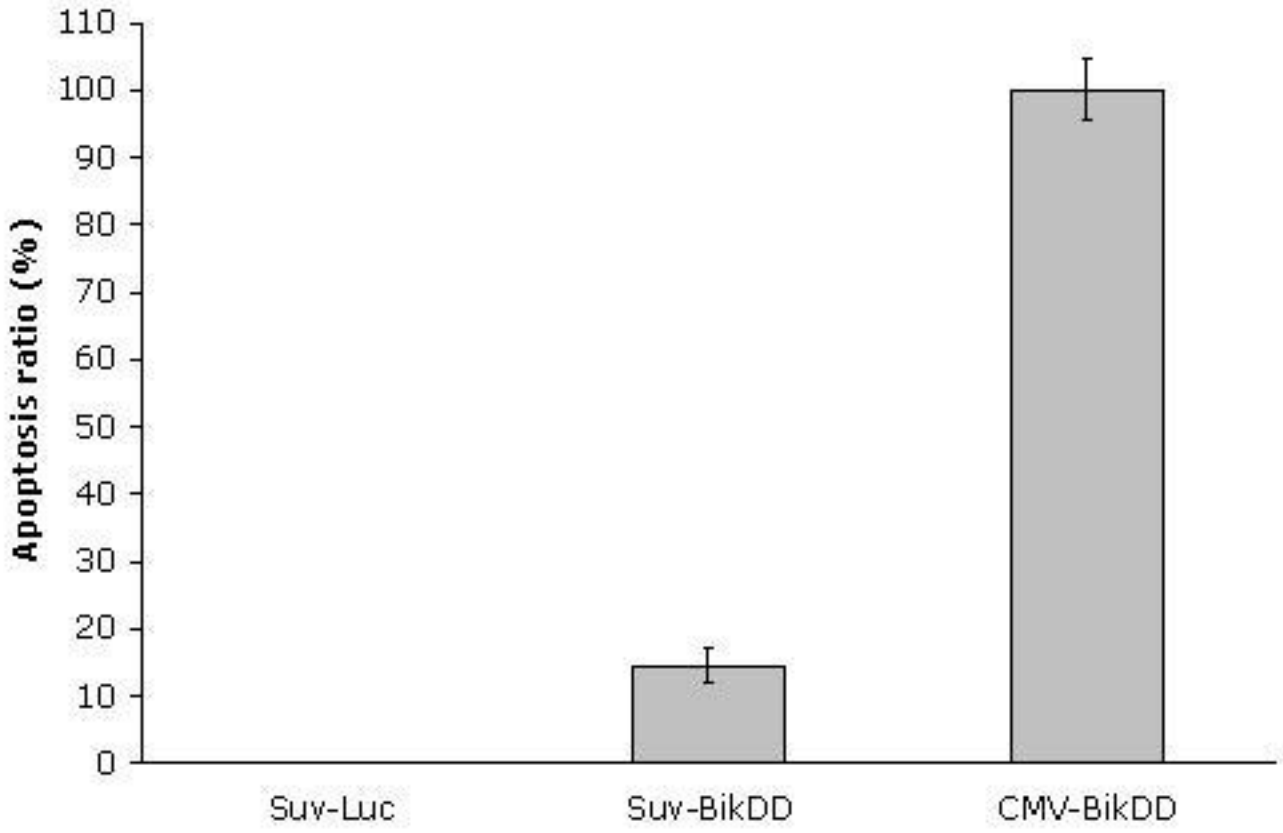
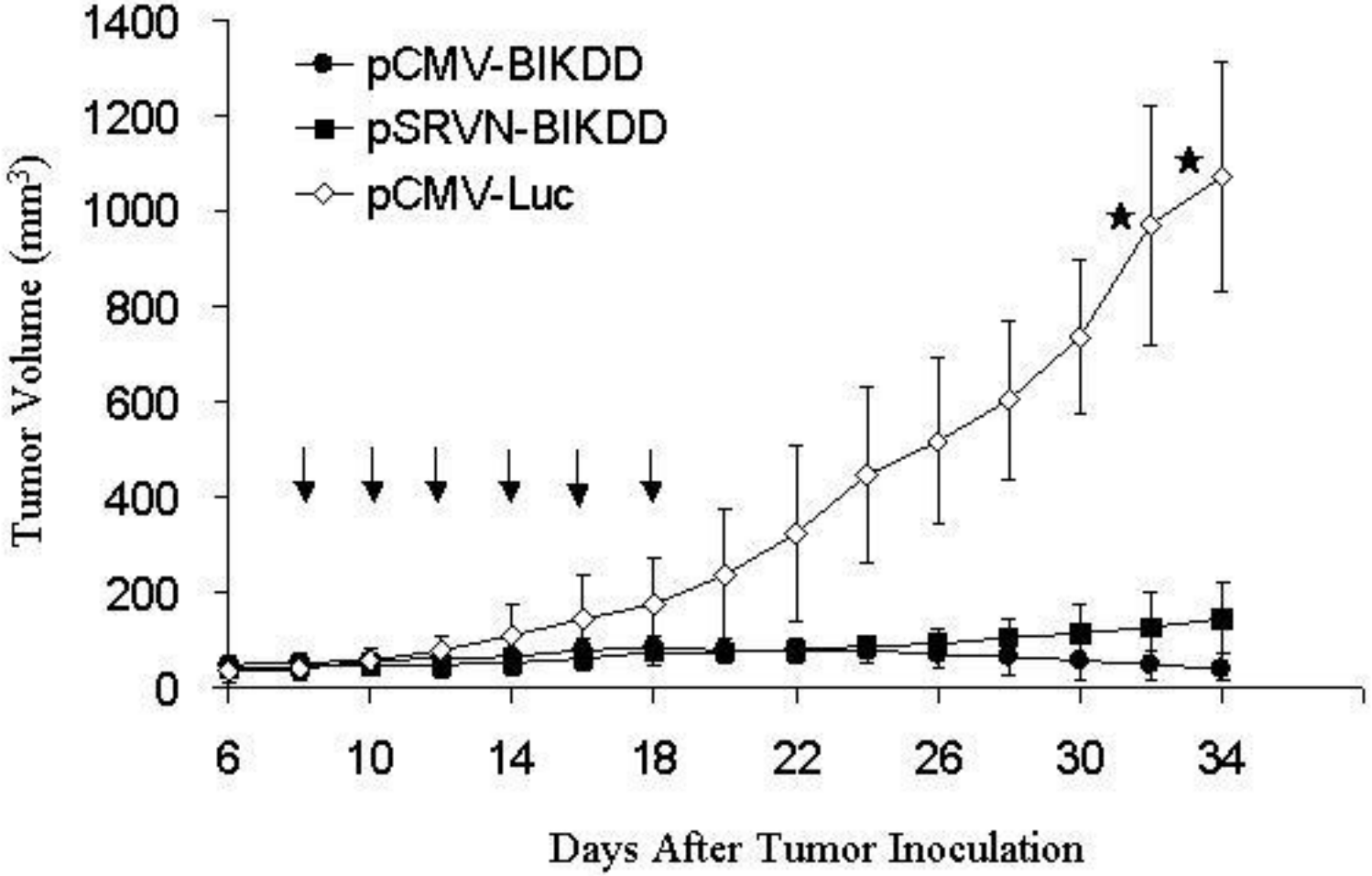

Apoptosis ratio (%)
110
100
90
80
70
60
50
40
30
20
10
0
Suv-Luc
Suv-BikDD
CMV-BikDD

Fig. 5E


1400
1200
1000
800
600
400
200
0
Tumor Volume (mm3)
pCMV-BIKDD
pSRVN-BIKDD
pCMV-Luc
6
10
14
18
22
26
30
34
Days After Tumor Inoculation