Inhibition of human lung adenocarcinoma growth using survivint34a by low-dose systematic administration

YAN SHAN[#], CHUNTING WANG[#*], LI YANG, LI JUAN CHEN, HONG XIN DENG, HAN SHUO YANG, ZHIMIAN LI, ZHIYONG LI, LI PAN, FEI LENG and YUQUAN WEI

State Key Laboratory of Biotherapy, West China Hospital, West China Medical School, Sichuan University, Keyuan Road 4, Chengdu, Sichuan, 610041, China

**Corresponding author (Fax, +86 28 85164060; Email, chtwang@163.com)*

[#]*These authors contributed equally to this research.*

Anti-apoptosis plays an important role in tumour formation and development. Survivin is a member of the inhibitor of apoptosis (IAP) family, which is a target for anti-cancer drug exploitation was replaced as development. We investigated the role of the homo dominant-negative mutant Survivin-T34A in suppressing human lung adenocarcinomas (A549). The anti-tumour activity of HSurvivinT34A plasmid was evaluated in the A549 cell line and nude mice bearing A549 subcutaneous tumours. Low-dose systemic administration was continuously used. The HSurvivinT34A plasmid (5 μg/one) complexed with a cationic liposome (DOTAP/Chol) significantly inhibited tumour growth in our model. We observed microvessel density degradation by CD31 immunohistochemistry and apoptotic cell increase by TUNEL assay, PI staining and flow cytometric analysis in the treated group. The present findings suggest that the HSurvivinT34A plasmid complexed with a cationic liposome may provide an effective approach to inhibit the growth of human lung adenocarcinomas *in vitro* and *in vivo*.

[Shan Y, Wang C, Yang L, Chen L J, Deng H X, Yang H S, Li Z, Li Z, Pan L, Leng F and Wei Y 2010 Inhibition of human lung adenocarcinoma growth using survivint34a by low-dose systematic administration; *J. Biosci.* **35** 209–216] **DOI** 10.1007/s12038-010-0025-3

1. Introduction

Lung cancer is considered to be the most common malignancy and a leading cause of death globally. Despite significant advances made during the past several decades in the treatment of lung cancer, the overall 5-year survival rate of patients is still low (Branko 2007).

Dysregulation of apoptosis is a common feature of malignant cells (Reed 1999) and represents a significant obstacle to the therapy of human cancer (Rudin and Thompson 1997). Among the regulators of apoptosis, interest has been recently focused on survivin (Altieri 2001), which is a member of the inhibitor of apoptosis (IAP) gene family. The most notable feature of the expression profile of survivin is that it is absent in most terminally differentiated normal tissues, while it is highly expressed in both malignancies and embryonic tissues (Deveraux and Reed 1999). As a bifunctional protein that regulates cell division and suppresses apoptosis (Altieri and Marchisio 1999), studies have shown that survivin is a prognostic indicator for poor survival in several malignancies (Chakravarti *et al.* 2002; Kennedy *et al.* 2003; Lu *et al.* 2004).

Recently, efforts to counteract survivin including antisense (Kanwar *et al.* 2001), ribozymes (Pennati *et al.* 2002), RNAi-mediation (Jiang *et al.* 2006), survivin knockdown, survivin-directed vaccines (Pisarev *et al.* 2003), or dominant negative mutants have proved that targeting survivin is a promising approach for cancer therapy. It has been suggested that dominant negative mutants, including survivin Thr34→Ala and Cys84→Ala, could reduce the proliferative potential of tumour cells and lead to caspase-dependent apoptosis (Grossman *et al.* 1999; 2001). Several

Keywords. Apoptosis; cationic liposome; human lung adenocarcinomas; survivin

Abbreviations used: ANOVA, analysis of variance; FBS, foetal bovine serum; H&E, haematoxylin and eosin; IAP, inhibitor of apoptosis; MVD, microvessel density; NSCLC, non-small cell lung cancer; PCR, polymerase chain reaction; PI, propidium iodine; P-S, penicillin/streptomycin; TAL, Tachypleus amebocyte lysate; TUNEL, terminal eoxynucleotidyltransferase-mediated dUTP nick-end labelling; VEGF, vascular endothelial growth factor

basic studies and phase I trials based on the administration of survivin-directed cytotoxic T lymphocytes have been completed and proved to be effective and safe to move on to clinical phase 2 studies (Pisarev *et al.* 2003; Tsuruma *et al.* 2004). In this study, we focus on the underexplored question of whether low-dose HSurvivinT34A plasmids (5 μg/one) complexed with cationic liposomes (DOTAP/Chol) would provide a positive anti-tumour effect on human lung adenocarcinomas by systemic administration.

2. Materials and methods

2.1 *Recombinant plasmids vector construction*

The recombinant plasmids carrying pORF9-HSurvivinT34A or pVITRO2-neo-mcs null were purchased from Invivogen Corporation,: San Diego, California (CA). HSurvivinT34A was amplified by polymerase chain reaction (PCR), using the forward primer 5′-CATCACGCGTCACCATGGGTGC-3′ and reverse primer 5′-CGGCGTCGACTCAATCCATAGCAG-3′ from pORF-9-HSurvivinT34A. After sequence confirmation, the recombinant plasmid was prepared using an Endofree Plasmid Giga kit (Qiagen, Chatsworth, CA). Endotoxin levels of the plasmid DNA prepared were determined by using Tachypleus amebocyte lysate (TAL). The plasmid PVITRO2-null was used as control.

2.2 *Cell lines and culture conditions*

The human lung carcinoma cell line A549 was obtained from the American Type Culture Collection (ATCC, Manassas, VA) and cultured in RPMI-1640 Medium (Gibco BRL, Grand Island, NY) supplemented with 10% heat-inactivated foetal bovine serum (FBS) and 1% penicillin/streptomycin (P-S). The cultures were maintained in a humidified chamber at 37°C in a 5% CO_2 atmosphere.

2.3 *Assessment of apoptosis* in vitro

The DOTAP:chol liposomes were prepared by our laboratory. The ideal plasmid:liposome ratio for the most efficient gene delivery is 1 μg:3 μg. For transfection, 3 × 10^5A549 cells were plated into 6-well plates (10 cm^2 growth area) and incubated overnight. Then 2 μg of DNA (HSurvivinT34A plasmid or PVITRO2 null plasmid) with 6 μl of liposomes were dissolved in 500 μl serum-free 1640 media without antibiotics for 30 min and added directly to the cell cultures. After 6 h, the media were replaced with fresh serum-supplemented media with antibiotics. Cells were harvested for apoptotic analysis 48 h later, as described below.

Samples were resuspended in propidium iodine (PI) and incubated in darkness for 5 min. Apoptosis was observed with fluorescence microscopy and by flow cytometry.

2.4 *Animals*

Female *BALB/C* nude (nu/nu) mice (6–8 weeks old) were purchased from the Laboratory Animal Center of Shanghai in China. They were maintained under pathogen-free conditions in our laboratory for animal experiments. All studies involving mice were approved by the Institutional Animal Care and Use Committee.

2.5 *Lung tumour xenograft models*

In our preliminary experiment, the animals were divided into four dose groups (1 μg, 2.5 μg, 5 μg, 10 μg) of HSurvivin T34A, with five mice per group. It was found that tumour growth was inhibited as much in the 5 μg group as in the 10 μg group. Tumour growth was significantly more inhibited in these two groups than in the 1 μg and 2.5 μg groups. Hence, we chose a 5 μg dose for our formal experiment. Female BALB/C nude (nu/nu) mice were injected subcutaneously with 5 × 10^6 of A549 cells in the right flank on day 0. Primary tumours had a mean diameter of about 3 mm on day 15. The tumour-bearing mice were randomly assigned to the following three groups and received the corresponding dose by injection: (a) NS group mice received 100 μl 0.9% NaCl solution (NS); (b) PVITRO2-null group mice received 5 μg PVITRO2-null plasmid/15 μg liposome complexes (volume =100 μl); and (c) HSurvivinT34A group mice received 5 μg HSurvivinT34A plasmid/15 μg liposome complexes (volume = 100 μl). Each group had 7 mice. We began systemic therapy on day 15 and repeated the injections daily for a total of 10 days. On day 38, all mice were euthanized. Tumour net weight of each mouse was measured. Tumour growth was evaluated by the measurement of tumour diameter every 2 or 3 days and the tumour volume was calculated as length × $width^2$ × 0.52. All the data are presented as mean ± SE. Normal organs and tumours from each mouse were removed and fixed in a 4% formaldehyde solution for histological analysis.

2.6 *Histology and immunohistochemistry*

Primary tumours were fixed in 4% paraformaldehyde, embedded in paraffin and cut into 3–5 μm sections. The sections were then stained with haematoxylin and eosin (H&E) for analysis.

CD31 immunohistochemistry was done for tumour microvessel density (MVD) according to the manual. Rabbit anti-human polyclonal CD31 (ab59251, Abcam company) was diluted 1/200 overnight at 4°C, followed

by incubation with biotinylated goat anti-rabbit IgG and streptavidin–biotin–horseradish peroxidase complex at 37°C for 1 h. Positive reactions were observed with 3,3′-diaminobenzidine (DAB substrate kit; Vector Laboratories) as a chromagen. Cellular nuclei were counterstained with ameliorative Gill haematoxylin. Quantification of MVD was assessed according to the method of Peng *et al.* (2008). At least three fields were counted, and the average was taken as the MVD of each section.

2.7 *Testing of the anti-apoptotic effect* in vivo

The presence of apoptotic cells within the tumour sections was evaluated by the terminal eoxynucleotidyltransferase-mediated dUTP nick-end labelling (TUNEL) technique using the DeadEnd Fluorometric TUNEL System (Promega, Madison, WI) according to the protocol. Per cent apoptosis was determined by counting the number of apoptotic cells and dividing by the total number of cells in the field (5 high power fields/slide).

2.8 *Statistical analysis*

The results of statistical analysis are presented as mean±SD. For comparison of condition at individual time points, differences between the groups were tested by performing analysis of variance (ANOVA) and unpaired Student *t* tests. All *P* values were two-sided and statistical significance was defined as $P<0.05$.

3. Results

3.1 *Anti-apoptotic effect of HSurvivinT34A plasmid* in vitro

Treatment with HSurvivinT34A plasmid contributed to morphological changes characteristic of apoptosis of A549 tumour cells: bright red-fluorescent nuclei (fragmented or intact) displayed by fluorescence microscopy of PI-stained nuclei, cell shrinkage, condensation of nuclear chromatin, nuclear fragmentation and apoptotic bodies (figure 1A). By contrast, the control (including the untransfected and the transfected with PVITRO2 null) did not demonstrate obvious changes. The average apoptotic numbers per field (×200) in every group was measured in at least five fields per slide (figure 1C, $*P<0.01$).

The quantitative assessment of sub-G1 cells by flow cytometry was used to estimate the number of apoptotic cells. A549 cells treated with HSurvivin T34A plasmid for 48 h had a striking sub-G1 peak compared with the untransfected group and the group transfected with PVITRO2 null plasmid (figure 1B), with 32.17%±0.87%, 5.4%±1.33% and 8.77%±0.93% sub-G1 cells (figure 1D,$*P<0.01$), respectively, as assessed by flow cytometry.

3.2 *Anti-tumour effect of HSurvivin T34A plasmid* in vivo

The established human lung adenocarcinoma (A549) xenograft model was used to observe the effect of HSurvivinT34A with cationic liposomes (DOTAP/chol) on the tumour-burdened mice. The treatment regimens were as described in Materials and methods. Tumours were monitored every 2–3 days (figure 2A). After day 23, there were statistical differences in the tumour volumes ($*P<0.05$) between the treatment group and the controls (including the NS group and PVITRO-2 null group). The inhibitory rate of the treatment group was up to 74.21% and the tumour volume of the vector group was up to about four times larger than that of the treated group (figure 2B). At the termination of the experiment, all the animals were euthanized and the tumour weights were measured (figure 2C). We also observed that the average weight of the tumours in the treated group was reduced by 67.95% when compared with the NS group ($*P<0.01$). The data showed that the HSurvivinT34A plasmid has a significant influence on the suppression of tumour growth. Meanwhile, no severe adverse consequences were detected by gross measures such as weight loss, ruffling of fur, and changes in behaviour and feeding. Furthermore, no pathological changes in the normal tissues such as heart, liver, spleen, lung or kidney were found by H&E staining (data not shown).

3.3 *Inhibition of tumour-induced angiogenesis (CD31) and increase of apoptosis (TUNEL)*

Tumour sections from each group were stained with H&E (figure 3A), anti-CD31 antibody and TUNEL reagent in order to evaluate the MVD and apoptosis rate as described above. Tumours in the control groups revealed a high MVD while those in the HSurvivinT34A group had lower values (figure 3B). In quantitative morphometric analysis, the treatment group had a ~3-fold suppression of the MVD: microvessel density in A549 xenografts as compared with the control (figure 3D, $*P<0.01$). In addition, there were large areas of necrosis and many more apoptotic cells in the treated group (figure 3C). Tumour tissues of the treated group tended to display the highest apoptotic indices at 25.82%±2.16%. The control group's indices were significantly lower, with the NS group's apoptotic index at 3.36%±0.62% and PVITRO-2 null group at 6.70%±0.76% (figure 3E, $*P<0.01$). These data suggest that the HSurvivinT34A plasmid could cause inhibition of angiogenesis and directly increase apoptosis of

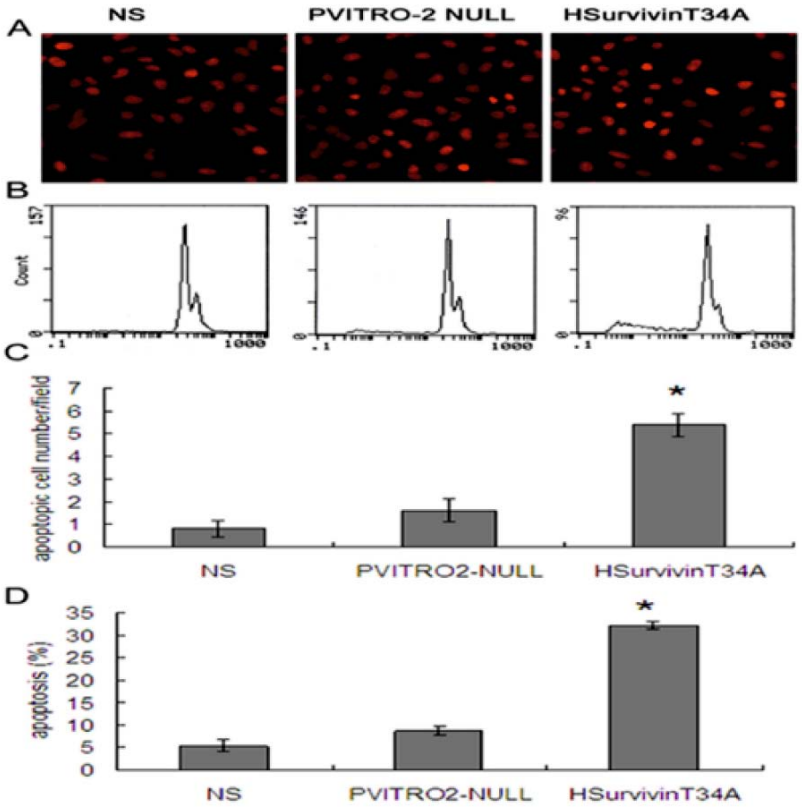

Figure 1. The anti-apoptotic effect of HSurvivinT34A plasmid *in vitro*. **(A)** Fluorescence microscopic appearance of propidium iodide (PI)-stained nuclei. A549 cells were treated with HSurvivinT34A plasmid (2 μg per well) for 48 h. After transfection with the HSurvivinT34A plasmid, reduction of cell volume, condensation of nucleus, nuclear fragmentation and apoptotic bodies is clear. The average apoptotic numbers per field (×200) were measured in at least five fields/slide (**C**, **P*<0.01). **(B)** The quantitative assessment of sub-G1 cells by flow cytometry was used to estimate the number of apoptotic cells. The average apoptosis percentage of three groups as assessed by flow cytometry was analysed. Columns, means; bars, SD. Compared with control groups, the HSurvivinT34A plasmid-treated cells showed a significant difference (**D**, **P* <0.01).

tumour cells, which plays an important role in the treatment of lung cancer.

4. Discussion

Lung cancer is the most common cause of cancer-related mortality globally.: Currently, adenocarcinoma is the most common histologyical type in both sexes, races, and in all age groups.

The IAP survivin deserves attention as a target for cancer therapy due to its differential expression in tumours versus normal tissues and as a bifunctional protein that acts as a suppressor of apoptosis and plays a central role in cell division (Altieri *et al.* 1999). Survivin may be an important prognostic marker of metastasis and proliferative potential (Mohamed *et al.* 2009) for patients with *non-small cell lung cancer* (NSCLC). Previous studies targeting survivin in lung cancer have mainly focused on inducing cell apoptosis (Shen *et al.* 2009; Mehdi *et al.* 2001; Blanc-Brude *et al.* 2003) and the sensitizing effects of radiotherapy (Bo *et al.* 2004) and chemotherapy (Robert *et al*. 2000) *in vitro*. There have been few trials *in vivo* to date. In our study, we evaluated the therapeutic efficacy of low-dose HSurvivinT34A plasmid on the A549 nude mice model by intravenous injection.

In our experiment, we found that HSurvivinT34A plasmid (5 μg)/DOTAP:chol complexes efficiently inhibited the growth of the human lung adenocarcinoma model by systemic administration. We observed that the average weight of tumours in the treated group was reduced by 67.95% compared with the controls. We believe that therapeutically targeting survivin produced significant results due to the following major mechanisms.

(i) *The induction of tumuor cell apoptosis:* The results of PI staining and flow cytometric analysis *in vitro* were the same as the results of TUNEL staining

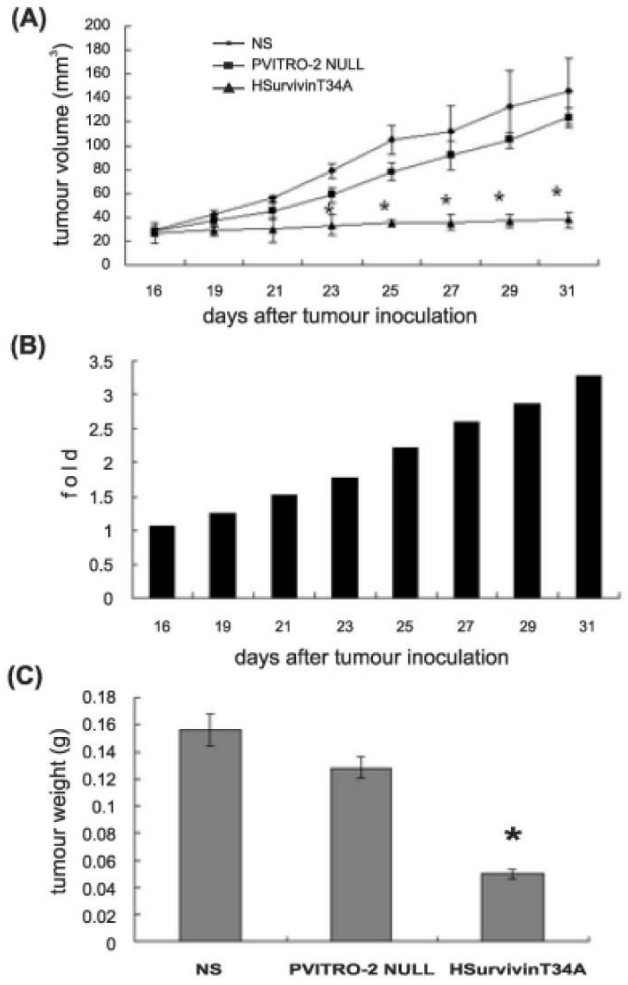

Figure 2. Anti-tumour effects of HSurvivinT34A plasmid: lipo complexes versus 0.9% NS, PVITRO2-null plasmid:lipo complexes. Data are presented as means ± SE. **(A)** Tumour volume was shown up to the day 31 after inoculation. The differences between HSurvivinT34A plasmid:lipo and control groups were significant (**P*<0.05) starting at day 23. **(B)** The comparison of tumour growth between mice injected with PVITRO2-null plasmid and mice treated with HSurvivnT34A plasmid. **(C)** Tumour weight was measured after all the animals were euthanized. Compared with that of the control groups, the average weight of the HSurvivinT34A plasmid-treated mice showed a significant difference (**P*<0.01).

in vivo. They suggest that HSurvivinT34A induced significantly more apoptotic cells in the A549 group than the control. These data are consistent with those from previous studies. Phosphorylation at Thr34 of survivin played an essential role in its regulation by cyclin-dependent kinase, p34cdc2-cyclinB1 (Muzio *et al.* 2001). Elevations in p34cdc2 kinase, induced by spindle checkpoint activation, resulted in increased survivin expression (Connor *et al.* 2002). The mutant survivin of Thr34-Ala (T34A) cannot be phosphorylated by p34cdc2-cyclinB1. It has been considered that the Thr34 phosphorylation site appears to be in a position to stabilize anti-apoptotic protein–protein interactions through the BIR domain of survivin (0: Verdecia *et al*. 2000). Failure to phosphorylate Thr34 leads to the dissociation of a survivin–caspase-9 complex and subsequent caspase-9-dependent apoptosis during cell division (Connor *et al.* 2000). Other researchers have detected that survivinT34A could be : worked asresponsible for a mitochondrial-dependent apoptosis with release of cytochrome c and loss of mitochondrial transmembrane potential by targeting caspase 9 and other molecules. Survivin could influence the upstream initiation of mitochondrial-dependent apoptosis. This should be considered a viable hypothesis, especially when one considers the association reported with Smac/DIABLO (Du *et al.* 2000), a mitochondrially released protein, which relieved the inhibitory function of IAP on caspase-9 activation (Shi 2002).

(ii) *The inhibition of angiogenesis:* The results of CD31 staining in each group detected that the MVD of the HSurvivinT34A plasmid group obviously decreased compared with the control. Research groups using a breast cancer model have also observed this phenomenon. Previous studies have demonstrated that this may involve upregulated survivin expression during the proliferative phase as well as the non-proliferative phase of angiogenesis as a transcriptional target of vascular endothelial growth factors (VEGF) (Connor *et al.* 2000). The remodelling of blood vessels is attributed to, among others, angiopoietin-1 (Harfouche *et al.* 2002; Papapetropoulos *et al.* 2000). Targeting of survivin resulted in the loss of cytoprotection afforded by VEGF against ceramide or tumour necrosis factor-*α*-induced apoptosis. This suggests that VEGF protects endothelial cells against apoptosis during angiogenesis by upregulating survivin (Mesri *et al.* 2001). Angiopoietin-1 has been shown to upregulate survivin in endothelial cells, via a PI3-K/AKT pathway, subsequently protecting them from apoptosis-inducing stimuli. However, interference by a dominant negative survivin resulted in the loss of angiopoietin-1. Thus, targeting survivin, in addition to inhibiting tumour cell growth, may also prove to be beneficial in inducing apoptosis in proliferating endothelial cells of the tumour vasculature.

The gene delivery system we utilized was cationic liposomes. This proved to efficiently increase the therapeutic

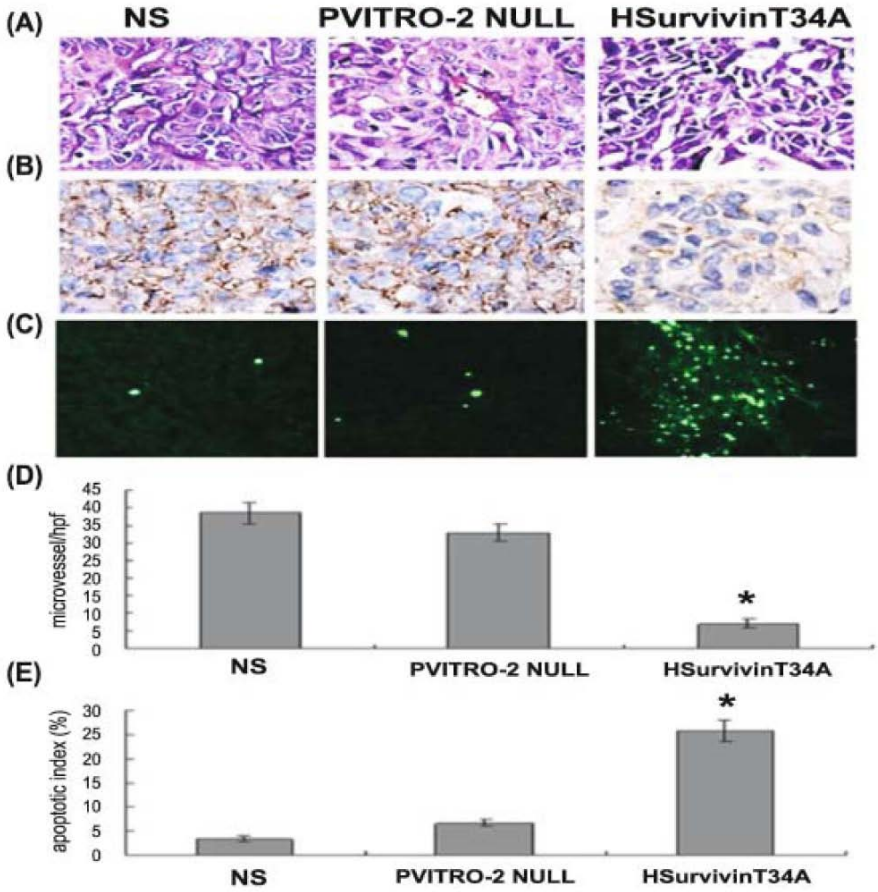

Figure 3. Inhibition of tumour-induced angiogenesis (CD31) and increase of apoptosis (TUNEL) (haematoxylin and eosin [H&E] stain). **(A)** Tumour sections from each group were stained with H&E. Representative sections from each group are shown (×400). **(B)** Inhibition of intratumoral angiogenesis assayed by CD31 staining of microvessels. Vascularization within tumours was detected by an antibody to CD31 and vascular density, which was quantified by counting the number of microvessels per high power field (×400). **(C)** Terminal deox ynucleotidyltransferase-mediated dUTP nick-end labelling (TUNEL) staining of tumour tissues. After treatment, sections were stained by TUNEL analysis to detect apoptotic cells. Per cent apoptosis was determined by counting the number of apoptotic cells and dividing by the total number of cells in the field (5 high power fields/slide). **(D)** CD31-positive microvessels in each group. Values are expressed as means ± SE (5 high power fields/slide). Tumours of the control groups (including NS and PVITRO2-null plasmid groups) demonstrated high microvessel density while those of the SurvivinT34A plasmid group had decreased values (**P*<0.01). **(E)** Per cent apoptosis in each group. Values are expressed as means ± SE. HSurvivinT34A plasmid:lipo complexes increased the per cent apoptosis in lung tumour sections relative to either normal saline or PVITRO2-neo-mcs null plasmid:lipo complexes (**P*<0.01).

efficacy of the HSurvivinT34A plasmid. We continuously employed a low-dose systemic therapy. Other research on DOTAP:chol-DNA complexes following intravenous administration indicate that DOTAP:chol liposomes can efficiently deliver genes to various organs and tissues with maximum delivery to the lung (Ito *et al.* 2003). Chol liposomes as a delivery system have increased uptake by tumour cells and angiogenic endothelial cells in tumours (Thurston *et al.* 1998). Repeated multiple treatments can increase gene expression when compared to single-dose treatment (Ramesh *et al.* 2001). Compared with intratumour injection, intravenous administration may additionally control micrometastases throughout the body and can be extended to clinical application with ease. The findings suggest that there is no other systemic injury through this route of administration and we feel this administration technique should be used for subsequent research.

In our laboratory, Peng *et al.* (2008) used SurvivinT34A plasmid (25 μg/one) complexed with cationic liposome (DOTAP:chol) by intravenous administration to cause anti-tumour effects in female BALB/c mice bearing 4T1 subcutaneous tumours, but in our preliminary tests, we found that HSurvivinT34A plasmid (5 μg/one) given in the human lung adenocarcinoma model had a similar therapeutic effect.

In this study, we have been able to demonstrate that the HSurvivin T34A plasmid complexed with cationic liposome delivered through low-dose systemic administration can efficiently inhibit the growth of human lung adenocarcinoma *in vitro* and *in vivo*. The potential positive therapeutic mechanisms derived from current hypotheses, which are confirmed by our data, are the triggering of apoptosis and inhibition of angiogenesis. Moreover, the positive therapeutic effect may be attributed to the autoimmune response stimulated by intravenous injection. In a clinical setting, gene therapy in the form of low-dose systemic administration can be effective, less toxic, affordable and convenient. Our findings may provide some reference for targeting survivin in lung cancer and suggest that the HSurvivinT34A plasmid can be considered as a feasible and effective treatment approach for lung cancer. We feel this trial provides a strong basis for further research into the effects of HSurvivinT34A plasmid by low-dose systemic administration in a clinical setting.

Acknowledgements

The authors thank members of the State Key Laboratory of Biotherapy for helpful discussions. The authors thank Adam P Allen for correcting the paper and Huashan Shi for technical assistance.

This work was supported by the National 973 Basic Research Program of China (2006CB504303 and 2006CB504302).

References

Altieri D C 2001 The molecular basis and potential role of survivin in cancer diagnosis and therapy; *Trends. Mol. Med*. **7** 542–547

Altieri D C and Marchisio C 1999 Survivin apoptosis: an interloper between cell death and cell proliferation in cancer; *Lab. Invest*. **79** 1327–1333

Blanc-Brude O P, Mesri M, Wall N R, Plescia J, Dohi T and Altieri D C 2003 Therapeutic targeting of the survivin pathway in cancer: initiation of mitochondrial apoptosis and suppression of tumor-associated angiogenesis; *Clin. Cancer Res*. **9** 2683–2692

Bo L, Yi M, Carolyn C, Fenghua Z, Sylke S, Jiahui T, Jim P, Jun C *et al.* 2004 Survivin as a therapeutic target for radiation sensitization in lung cancer; *Cancer Res*. **64** 2840–2845

Branko Z 2007 *Report 2007* 12th World Conference on Lung Cancer. Seoul, Korea.

Chakravarti A, Noll E, Black P M, Finkelstein D F, Finkelstein D M, Dyson N J and Loeffler J S 2002 Quantitatively determined survivin expression levels are of prognostic value in human gliomas; *J. Clin. Oncol*. **20** 1063–1068

Connor D S, Grossman G, Plescia J, Li F, Zhang H, Villa A, Tognin S, Marchisio P C *et al.* 2000 Regulation of apoptosis at cell division by p34cdc2 phosphorylation of survivin; *Proc. Natl. Acad. Sci. USA* **97** 13103–13107

Connor D S, Schechner J S, Adida C, Mesri M, Rothermel A L, Li F, Nath A K, Pober J S *et al.* 2000 Control of apoptosis during angiogenesis by survivin expression in endothelial cells; *Am. J. Pathol*. **156** 393–398

Connor D S, Wall N R, Porter A C G and Altieri D C 2002 A p34cdc2 survival checkpoint in cancer; *Cancer Cell* **2** 43–54

Deveraux Q L and Reed J C 1999 IAP family proteins: suppressors of apoptosis; *Genes. Dev*. **13** 239–252

Du C, Fang M, Li Y, Li L and Wang X 2000 Smac, a mitochondrial protein that promotes cytochrome c-dependent caspase activation by eliminating IAP inhibition; *Cell* **102** 33–42

Grossman D, Kim P J, Schechner J S and Altieri D C 2001 Inhibition of melanoma tumor growth in vivo by survivin targeting; *Proc. Natl. Acad. Sci. USA* **98** 635–640

Grossman D, McNiff J M, Li F and Altieri D C 1999 Expression and targeting of the apoptosis inhibitor, survivin, in human melanoma; *J. Invest. Dermatol*. **113** 1076–1081

Harfouche R, Hassessian H M, Guo Y, Faivre V, Srikant C B, Yancopoulos G D and Hussain S N 2002 Mechanisms which mediate the antiapoptotic effects of angiopoietin-1 on endothelial cells; *Microvasc. Res*. **64** 135–147

Ito I, Began G, Mohiuddin I, Saeki T, Saito Y, Branch C D, Vaporciyan A, Stephens L C *et al*. 2003 Increased uptake of liposome-DNA complexes by lung metastases following intravenous administration; *Mol. Ther*. **7** 409–418

Jiang G, Li J, Zeng Z and Xian L 2006 Lentivirus-mediated gene therapy by suppressing survivin in BALB/c nude mice bearing oral squamous cell carcinoma; *Cancer Biol. Ther*. **5** 435–440

Kanwar J R, Shen W P, Kanwar R K, Berg R W and Krissansen G W 2001 Effects of survivin antagonists on growth of established tumors and B7-1 immunogene therapy; *J. Natl. Cancer Inst.* **93** 1541–1552

Kennedy S M, Driscoll L, Purcell R, Fitz-Simons N, McDermott E W, Hill A D, Higgins N J, Parkinson M *et al.* 2003 Prognostic importance of survivin in breast cancer; *Br. J. Cancer* **88** 1077–1083

Lu B, Gonzalez A, Massion P P, Shyr Y, Shaktour B, Carbone D P and Hallahan D E 2004 Nuclear survivin as a biomarker for non-small-cell lung cancer; *Br. J. Cancer* **91** 537–540

Mehdi M, Nathan R, Jia L, Richard W. Kim and Dario C 2001 Cancer gene therapy using a survivin mutant adenovirus; *J. Clin. Invest*. **108** 981–990

Mesri M, Morales-Ruiz M, Ackermann E J, Bennett C F, Pober J S, Sessa W C and Altieri D C 2001 Suppression of vascular endothelial growth factor-mediated endothelial cell protection by survivin targeting; *Am. J. Pathol*. **158** 1757–1765

Mohamed S, Yasufuku K, Nakajima T, Hiroshima K, Chiyo M, Sekine Y, Shibuya K, Agamy G *et al*. 2009 Nuclear survivin in pN2 nonsmall cell lung cancer: prognostic and clinical implications; *Eur. Respir*. **33** 127–133

Muzio L, Staibano S, Pannone G, Mignogna M D, Mariggio A, Salvatore G, Chieffi P, Tramontano D *et al.* 2001 Expression of the apoptosis inhibitor survivin in aggressive squamous cell carcinoma; *Exp. Mol. Pathol*. **70** 249–254

Papapetropoulos A, Fulton D, Mahboubi K, Kalb R G, Connor D S, Li F, Altieri D C and Sessa W C 2000 Angiopoietin-1 inhibits

endothelial cell apoptosis via the Akt/survivin pathway; *J. Biol. Chem*. **275** 9102–9105

Peng X C, Yang L, Yang L P, Mao Y Q, Yang H S, Liu J Y, Zhang D M, Chen L J *et al.* 2008 Efficient inhibition of murine breast cancer growth and metastasis by gene transferred mouse survivin Thr34→Ala mutant; *J. Exp. Clin. Cancer Res*. **27** 46–59

Pennati M, Colella G, Folini M, Citti L, Daidone M G and Zaffaroni N 2002 Ribozyme-mediated attenuation of survivin expression sensitizes human melanoma cells to cisplatin-induced apoptosis; *J. Clin. Invest*. **109** 285–286

Pisarev V, Yu B, Salup R, Sherman S and Gabrilovich D I 2003 Full-length dominant-negative survivin for cancer immunotherapy; *Clin. Cancer Res*. **9** 6523–6533

Ramesh R, Saeki T, Templeton N S, Ji L, Stephens L C, Ito I, Wilson D R, Wu Z *et al*. 2001 Successful treatment of primary and disseminated human lung cancers by systemic delivery of tumor suppressor genes using an improved liposome vector; *Mol. Ther*. **22** 11–16

Reed J C 1999 Dysregulation of apoptosis in cancer; *J. Clin. Oncol*. **17** 2941–2953

Robert A, Olie A, Paula S, Bettina B, Sian H L, Doriano F, Rolf A and Stahel U 2000 A Novel antisense oligonucleotide targeting survivin expression induces apoptosis and sensitizes lung cancer cells to chemotherapy; *Cancer Res*. **60** 2805–2809

Rudin C M and Thompson C B 1997 Apoptosis and disease: regulation and clinical relevance of programmed cell death; *Annu. Rev. Med*. **48** 267–281

Shen C X, Liu W J, Andreas K B and Sven N R 2009 Pro-apoptosis and anti-proliferation effects of a recombinant dominant-negative survivin-t34a in human cancer cells; *Anticancer Res.* **29** 1423–1428

Shi Y 2002 Mechanisms of caspase activation and inhibition during apoptosis; *Mol. Cell*. **9** 459–470

Thurston G, Mclean J W, Rizen M, Baluk P, Haskell A, Murphy T J, Hanahan D and McDonald D M 1998 Cationic liposome target angiogenic endothelial cells in tumors and chronic inflammation in mice; *J. Clin. Invest*. **101** 1401–1413

Tsuruma T, Hata F, Torigoe T, Furuhata T, Idenoue S, Kurotaki T, Yamamoto M, Yagihashi A *et al*. 2004 Phase I clinical study of antiapoptosis protein, surviving derived peptide vaccine therapy for patients with advanced or recurrent colorectal cancer; *J. Transl. Med*. **2** 19–30

Verdecia M A, Huang H, Dutil E, Kaiser D A, Hunter T and Noel J P 2000 Structure of the human anti-apoptotic protein survivin reveals a dimeric arrangement; *Nat. Struct. Biol*. **7** 602–608

MS received 26 February 2010; accepted 29 April 2010

*e*Publication: 11 May 2010

Corresponding editor: Rita Mulherkar