

# Knockdown of survivin expression by siRNAs enhances chemosensitivity of prostate cancer cells and attenuates its tumorigenicity

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Survivin, a member of inhibitor of apoptosis family protein, has become an attractive therapeutic target in cancer due to its selective expression in tumor cells and its important roles for tumor cell viability. Here, we show that vector-based small interfering RNAs (siRNAs) silenced survivin expression in prostate cancer cells, resulting in significantly reduced cell proliferation and enhanced apoptosis, and increased the sensitivity of prostate cancer cells (PC-3) to the apoptosis-inducing agent, platinol. Furthermore, PC-3 cells transfected with the siRNA-expressing vector showed lower tumor formation in nude mice xenografts in vivo. These results demonstrated that inhibition of survivin expression by siRNA attenuated the malignant phenotypes of prostate cancer cells, and may provide a novel approach for gene therapy of androgen-independent prostate cancer.

*Keywords* RNA interference; survivin; prostate cancer; apoptosis; gene therapy

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# Introduction

Increases in the susceptibility to chemotherapy would represent an important advancement in the treatment of patients with advanced and metastatic prostate cancer [1]. Survivin is a 16.5 kDa intracellular protein with 142 amino acid residues and belongs to the inhibitor of apoptosis (IAP) family [2]. It is a bifunctional member of the IAP family that counteracts cell death and controls mitotic progression [3]. Survivin is present during embryonic development, but becomes undetectable in most adult tissues [4]. In nearly all human cancers examined so far, survivin expression is re-activated [5-7]. Predictive and prognostic significances of survivin have been implemented in some types of cancer [8–13]. In general, two considerations have made survivin an attractive therapeutic target in cancer, namely, its selective expression in tumor cells and its important roles for tumor cell viability [14].

In recent years, considerable efforts have been made to develop strategies for modulating apoptosis in cells of cancer and other human diseases [15]. In this context, approaches to counteract survivin in tumor cells have been proposed, with the dual aims to inhibit tumor growth through an increase in spontaneous apoptosis and to enhance tumor cell responsiveness to apoptosis-inducing agents [16]. To date, different kinds of survivin antagonists, including antisense oligonucleotides, ribozymes, small interfering RNAs (siRNAs), and dominantnegative mutants, as well as cyclin-dependent kinase inhibitors, have been tested [17].

RNA interference (RNAi) is a genetic interference phenomenon directed by double-stranded RNA. RNAi specifically and efficiently degrades homogeneous mRNA, resulting in post-transcriptional gene silencing [18,19]. Since its discovery in 1998, RNAi has been rapidly developed into one of the most widely applied technologies with therapeutic potential in molecular and cellular research [20,21].

To explore the feasibility of survivin-targeted siRNAs therapy for prostate cancer, the expression of survivin in androgen-independent prostate cancer cell lines PC-3, PC-3M, and DU145, and androgen-dependent prostate cancer cell lines LNCaP and 22RV1 were investigated. Survivin-targeted siRNAs expression vectors were constructed and androgen-independent prostate cancer cell line PC-3 was transfected with the vector-based siRNA.

Next, the biological effects of survivin knockdown on cell chemosensitivity and tumorigenicity were observed *in vitro* and *in vivo*. These experiments lay a substantial foundation for understanding the function of survivin and gene therapy of human androgen-independent prostate cancer.

# Materials and methods

### Materials

pSilencer3.1-H1neo- and pSilencer3.1-H1neo-negative control vectors were purchased from Ambion Inc. (Austin, USA). RPMI-1640 medium and Trizol reagent were purchased from Gibco-BRL (Life Technologies, Inc., Grand Island, USA). *Taq* DNA polymerase and dNTPs were obtained from Promega (Madison, USA). DNA marker DL-15000, T4 DNA ligase, *Bam*HI, and *Hind*III were bought from Takara (Dalian, China). Lipofectamine 2000<sup>TM</sup> and reverse transcription kit were purchased from Invitrogen (Carlsbad, USA). Polyclonal rabbit anti-human survivin antibodies, polyclonal rabbit anti- $\beta$ -actin antibodies, and horseradish peroxidase-conjugated goat anti-rabbit IgG antibody were purchased from Maxim Biological Co. (Fuzhou, China).

### Cell lines and culture

The human androgen-independent prostate cancer cell lines PC-3, PC-3M, and DU145, and androgendependent prostate cancer cell lines LNCaP and 22RV1 were maintained in our laboratory and were grown as monolayer in RPMI-1640 media containing 10% fetal bovine serum (FBS) at  $37^{\circ}$ C in a humidified 5% CO<sub>2</sub> incubator.

#### Immunocytochemical staining

Immunocytochemical staining was performed using the streptavidin biotin complex (SABC) method with an SABC kit (Boster, Wuhan, China). Adherent cells were treated with 3 ml/l H<sub>2</sub>O<sub>2</sub> in methanol for 30 min to bleach the endogenous peroxidase activity. Samples were blocked with normal goat serum for 30 min at room temperature, followed by incubating with the rabbit anti-human survivin antibody (1:200) overnight at 4°C. The samples were then incubated with the anti-rabbit IgG antibody and were developed in a substrate solution of 0.1 ml/l diaminobenzidine-hydrogen peroxide, followed by counterstaining with hematoxylin. Tumor cell immunostaining was scored by: the percentage of immunopositive cells  $(0-100) \times$  staining intensity grades (0, 1, 2, or 3) [22].

# Construction of plasmids expressing short hairpin RNAs (shRNAs)

Three siRNA sequences targeting the human survivin, including SVV1, 5'-GGACCACCGCATCTCTACA-3'; SVV2, 5'-GAAGCAGTTTGAAGAATTA-3'; and SVV3, 5'-GAATTAACCCTTGGTGAAT-3', were designed using the siRNA target finder tool (http://www.ambion.com/ techlib/misc/siRNA\_finder.html). The sense and antisense oligonucleotide chains expressing siRNA were synthesized (Sangon, Shanghai, China) by established methods with sequences as described previously [23]. Each siRNA gene fragment was flanked with a BamHI site at 5' end and a HindIII site at 3' end for cloning. Vectors expressing siRNA were designated as pSilencer3.1-SVV1, pSilencer3.1-SVV2, and pSilencer3.1-SVV3, respectively. The pSilencer3.1-H1neo-negative control vector was used as siRNA-negative control.

#### **Transfection of PC-3 cells**

Before transfection, cells  $(1 \times 10^{5}/\text{well})$  were seeded in 6-well plates and grown to 90–95% of confluence. Prior to transfection, the medium was replaced by serum-free RPMI-1640 (2000 µl/well, without antibiotics). Serumfree RPMI-1640 (250 µl) containing 2 µg plasmid was mixed with 250 µl serum-free RPMI-1640 containing 5 µl Lipofectamine 2000<sup>TM</sup> reagents. The resultant mixture was kept at room temperature for 30 min, and then was added into the plates, 500 µl each well. The plates were incubated at 37°C for 4 h, and FBS (500 µl) was added into each well. Cells were then cultured for an additional 48 h before further analysis. The efficiency of transfection was monitored by the pEGFP-N2 vector (Promega) under IX71 inverted fluorescence microscope (Olympus, Japan).

# Reverse transcription–PCR and calculation of relative *survivin* mRNA levels

Cellular RNA was isolated from transfected PC-3 cells using the Trizol reagent according to the manufacturer's instructions. Total RNA (1.0 µg) was reverse-transcribed into cDNA using a reverse transcription kit. The resultant cDNA was amplified using primers for the human *survivin*: sense, 5'-CTTTCTCAAGGACCACCGCATCT -3'; and antisense, 5'-GCACTTTCTCCGCAGTTTCCT C-3', which produced a 359-bp fragment. Cycling conditions were: initial denaturation at 95°C for 2 min, followed by 25 cycles at 95°C for 1 min, 58°C for 30 s, 72°C for 30 s, and 72°C for 7 min. A  $\beta$ -actin fragment (353-bp) was amplified and used as an internal control for the PCR. The amplified products were analyzed by 1.5% agarose gel electrophoresis and semi-quantified using an image analysis system (FR200; Furi, Shanghai, China). The *survivin* mRNA levels from cells transfected with pSilencer3.1-SVV or with pSilencer3.1-NC empty vector were first normalized against  $\beta$ -actin. Then, the normalized *survivin* mRNA levels from pSilencer3.1-SVV-transfected cells were plotted as the percentage of expression relative to those from pSilencer3.1-NCtransfected cells.

#### Western blot analysis

The transfected PC-3 cells were washed with PBS and lysed at 4°C for 30 min in PBS containing 50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1.0% Triton X-100, 0.1% SDS, 10% glycerin, and 1.0 mM phenylmethyl sulfonylfluoride. Cell extracts were centrifuged at 12,000 g for 20 min at 4°C, and the supernatants (containing up to 300 µg of total proteins) were mixed with an equal volume of  $2 \times$  SDS loading buffer. Samples were heated at 95°C for 5 min and were separated by SDS-15% PAGE and electrotransferred to nitrocellulose filters. The filters were blocked in PBS containing 5% skimmed milk and incubated overnight with the primary antibody specific for survivin, and then incubated with the secondary horseradish peroxidase-conjugated antibody. Bound antibody was detected using the substrate solution of 0.1 ml/l diaminobenzidine-hydrogen peroxide. An anti- $\beta$ -actin monoclonal antibody was used as an internal control. The results were semi-quantified using an image analysis system and the relative survivin protein levels were figured out with the similar methods used in calculation of the relative survivin mRNA levels.

#### Flow cytometry (FCM)

To examine cell apoptosis, transfected cells were fixed in 70% (v/v) ethanol, stained with an ANNEXIN V-FITC apoptosis assay kit (Becton–Dickinson, San Jose, USA) according to the instruction manual, and analyzed with a flow cytometer (FACSCalibur<sup>TM</sup>, Becton– Dickinson). In some experiments, transfected cells were treated with 5.0  $\mu$ g/ml platinol and were cultured for an additional 48 h prior to the flow cytometry (FCM) analysis.

For cell cycle analysis, transfected cells were stained with a CycleTEST<sup>TM</sup> PLUS DNA cell cycle assay kit (Becton–Dickinson) and then analyzed by FCM.

## MTT assay

Transfected cells  $(2 \times 10^4)$  were seeded in 24-well plates and cultured in the presence of MTT [3-(4,5-

dimethylthiazol-2-yl)2,5-diphenyltetrazolium bromide]. At each time point, cells were harvested, lysed, and the absorbance (*A*) at 490 nm of the supernatants was measured using a Microplate Reader (Becton–Dickinson). Cell number was calculated by comparison with a standard curve of known number of cells. In some experiments, transfected cells were treated with platinol ( $5.0 \mu g/ml$ ) before the analysis.

#### In vivo inoculation of tumors

Nude mice (eight for each group) were injected subcutaneously with 0.5 ml  $(2.0 \times 10^7/\text{ml})$  PC-3 cells transfected with pSilencer3.1-SVV1, pSilencer3.1-SVV2, pSilencer3.1-SVV3, or pSilencer3.1-H1neo-negative control vectors. Tumor volumes were measured every day. Four weeks later, mice were sacrificed and tumor weights were measured.

#### Statistical analysis

Data were expressed as mean  $\pm$  SD. Statistical significance was determined by the Student's *t*-test and the SNK-*q* test. A value of *P* < 0.05 was considered as statistically significant.

### Results

#### Expression of survivin in prostate cancer cell lines

We first examined the survivin expression in prostate cancer cell lines by immunohistochemistry. The results showed that the proportion of survivin-positive cells were 23% in PC3, 18% in DU145, 15% in LNCaP, 11% in 22RV1, and 12% in PC3M [Fig. 1(A)]. Immunoscores for PC3, PC3M, DU145, LNCaP, and 22RV1 were 49.42  $\pm$ 4.71,  $28.45 \pm 6.82$ ,  $34.27 \pm 6.08$ ,  $34.09 \pm 5.22$ , and  $31.66 \pm 7.13$ , respectively. The highest expression level of survivin was observed in the PC3 cells, compared with PC3M, DU145, LNCaP, and 22RV1. RT-PCR showed that the mRNA of survivin was expressed in five prostate cancer cell lines, which was confirmed by western blot, with the expression level higher in PC-3 and LNCaP cells [Fig. 1(B,C)]. Due to the highest expression of survivin in PC-3 cells among all tested prostate cancer cells, PC-3 was chosen as model cell in the following experiments.

# Silencing of survivin by RNAi inhibits cell proliferation and enhances chemosensitivity of prostate cancer cells

The efficiency of transfection was  $\sim 75\%$  as determined by pEGFP-N2 vectors [Fig. 2(A)]. To study the functions of survivin in prostate cancer cells, we employed the



**Fig. 1 Expression of survivin in prostate cancer cell lines** (A) The immunocytochemical staining of survivin in prostate cancer cell lines. (a) PC-3, (b) PC-3M, (c) DU145, (d) LNCaP, (e) 22RV1, and (f) negative control staining of survivin in PC-3M cells; magnitude,  $\times 200$ . (B) RT– PCR of *survivin* mRNA in prostate cancer cell lines. (C) Western blot analysis of survivin in prostate cancer cell lines.  $\beta$ -Actin was used as control.

RNAi strategy. PC-3 cells were transfected with siRNAexpressing vectors, and survivin mRNA and protein were measured by RT–PCR and western blot, respectively. The results indicated that pSilencer3.1-SVV2 and pSilencer3.1-SVV3 vectors were able to knockdown the expression of survivin [**Fig. 2(B,C)**]. The apoptosis index of PC-3 cells transfected with pSilencer3.1-SVV2 and pSilencer3.1-SVV3 increased greatly [**Fig. 3(A)**], whereas the proliferation of these cells decreased  $\sim$ 30%, as described previously [23]. In contrast with the control groups, the number of cells in the G<sub>1</sub> phases of the siRNA group increased 10%, and cells in the G<sub>2</sub> and S phases decreased  $\sim$ 5% [**Fig. 3(B**)]. Moreover, after treatment with platinol, the survival of the siRNA vector-transfected PC-3 cells decreased  $\sim$ 35–45% [**Fig. 3(C)**], whereas the apoptosis index increased  $\sim$ 29–42%, compared with control groups [**Fig. 3(A)**].

# Knockdown of survivin in PC-3 cells attenuates its tumorigenicity in nude mice xenografts

To examine the effect of survivin on tumor formation by prostate cancer cells, we performed a tumorogenesis assay in nude mice. Nude mice were inoculated subcutaneously with PC-3 cells transfected with pSilencer3.1-SVV1, pSilencer3.1-SVV2, or pSilencer3.1-SVV3 vectors, respectively. Four weeks later, the mean volume and weight of tumors examined. The results showed that the volume and weight of the tumors derived from PC-3 cells



**Fig. 2 Transfection efficiency and suvivin silencing at mRNA and protein level by vector-based RNAi** (A) Transfection efficiency of PC-3 cells. (B) The relative *survivin* mRNA levels in PC-3 cells 24 h after transfection with various siRNA expression plasmids. (C) The relative survivin protein levels in PC-3 cells 72 h after transfection with various siRNA expression plasmids.



**Fig. 3 Survivin silencing declined cell proliferation and enhanced chemosensitivity of prostate cancer cells** (A) Apoptosis index of PC-3 cells treated with siRNA and platinol. (B) Cells cycle analysis of PC-3 cells transfected with siRNA expression plasmids. (C) Cell viability of PC-3 cells transfected with siRNA expression plasmids and treated with platinol.

transfected with pSilencer3.1-SVV2 and pSilencer3.1-SVV3 vectors were 50-55% of those of the control groups (**Fig. 4**). These results indicate that the tumor formation and growth in nude mice xenografts were suppressed by silencing of survivin.

# Discussion

Survivin is an anti-apoptotic protein, which is also involved in mitotic checkpoint control and apoptosis induced by growth factor in human cancer cells [24]. Survivin is overexpressed in a variety of human neoplasms. Currently, survivin expression has been used as a prognostic factor in several human neoplasms. High survivin expression by neoplasms correlates with more aggressive behavior, decreased responsiveness to chemotherapeutic agents, and shortened survival, when compared with cancers that are survivin-negative [25].

Recently, Zhang *et al.* [22] have shown that survivin mediates resistance to anti-androgen therapy with flutamide in prostate cancer cells. Specifically, these authors suggested that up-regulation of survivin via insulin-like



Fig. 4 Survivin knockdown in PC-3 cells suppressed tumor formation in nude mice xenografts (A) The growth curve of tumor in nude mice bearing prostate cancer cell xenografts. (B) Tumors in nude mice bearing prostate cancer cell xenografts. (C) The tumor weight in nude mice bearing prostate cancer cell xenografts.

growth factor-1/AKR T-cell lymphoma (AKT) signaling during androgen blockade may be one of the mechanisms by which prostate cancer cells develop resistance to anti-androgens. In addition, survivin expression can be induced by heavy ion beams in some cancer cells, although the direct killing effect on cancerous cells by heavy ion radiation might be more significant than the anti-apoptosis effects of the survivin overexpression induced [26]. Overall, the results obtained in different

studies indicate survivin to be a cellular factor potentially involved in the chemo-resistant and radiation-resistant phenotypes of human tumors cells and suggest that approaches designed to inhibit survivin expression may lead to human tumor sensitization to chemical and physical agents. McEleny et al. [27] found that the androgenindependent PC-3 and DU145 cells were highly resistant to apoptosis and expressed the highest levels of NAIP, XIAP, and survivin mRNA. Furthermore, Krajewska et al. [28] found that high expression of these IAP family proteins was evident in human prostate cancers and in prostate tissues from transgenic mice expressing SV40 large T antigen under the control of a probasin promoter. Positive survivin immunostaining was found in many invasive cancers but was not detected in normal prostate epithelium. Interestingly, positive immunostaining was found in occasional non-transformed prostatic epithelial cells of both transgenic mice and normal mice, associated with chromosomes or mitotic structures in cells apparently undergoing division.

In order to knockdown the overexpression of survivin in cancer cells, various strategies have been investigated. Multiple hammerhead ribozymes targeting the exposed regions of survivin mRNA synergistically produced the most potent anti-cancer effects and inhibited tumor growth in a hepatocellular carcinoma xenograft mouse model [29]. Uchida et al. [30] developed recombinant adenoviral vectors expressing siRNA against the survivin transcript and explored the impact of these vectors on three cancer cell lines. In all cell lines tested, knockdown of the survivin expression resulted in apoptotic cell death. Cancer cells infected with these vectors almost lost their tumorigenicity, following inoculation into nude mice. Intratumoral injection with the vectors significantly suppressed tumor growth in a mouse xenograft model. This novel strategy may be a promising tool for cancer gene therapy. Yang et al. [14] also showed that in the absence of survivin, certain euploid human cells undergo mis-segregation of chromosomes, abortive assembly of microtubules in late mitosis, failure of cytokinesis, and arrest of DNA synthesis. These results further indicate the significances of survivin in cancer.

In a word, survivin is highly expressed in the PC-3, LNCAP, and DU145 prostate cancer cells. The two pSilencer3.1-SVV2 and pSilencer3.1-SVV3 constructs could knockdown the expression of survivin in a similar manner [31]. PC-3 cells transfected with the siRNA expression vectors showed increased apoptosis, decreased proliferation, and increased sensitivity to the proapoptotic stimuli platinol. The tumorogenesis activity

of these cells also decreased. These results are important in revealing the role of survivin in prostate cancer [32]. Due to the poor transfection efficiency, the mRNA and protein levels of survivin in prostate cancer cells could not be knocked down obviously enough in the experiments, further methods such as adenoviral or lentiviral delivery system can be used to overcome this disadvantage. A full understanding of effects of survivin in prostate cancer may lead to the development of new treatments for metastatic prostate cancer. Taken together, these findings indicate that survivin is significantly expressed in prostate cancer cells. Silencing of survivin expression by RNAi attenuated the malignant phenotypes of prostate cancer cells and may provide a novel approach for gene therapy of androgen-independent prostate cancer.

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# References

- 1 Zhang M, Latham DE, Delaney MA and Chakravarti A. Survivin mediates resistance to antiandrogen therapy in prostate cancer. Oncogene 2005, 24: 2474–2482.
- 2 Ambrosini G, Adida C and Altieri DC. A novel anti-apoptosis gene, survivin, expressed in cancer and lymphoma. Nat Med 1997, 3: 917–921.
- 3 Altieri DC and Marchisio PC. Survivin apoptosis: an interloper between cell death, cell proliferation in cancer. Lab Invest 1999, 79: 1327–1333.
- 4 Kim PJ, Plescia J, Clevers H, Fearon ER and Altieri DC. Survivin and molecular pathogenesis of colorectal cancer. Lancet 2003, 362: 205–209.
- 5 Adida C, Crotty PL, McGrath J, Berrebi D, Diebold J and Altieri DC. Developmentally regulated expression of the novel cancer antiapoptosis gene survivin in human and mouse differentiation. Am J Pathol 1998, 152: 43–49.
- 6 Li F and Ling X. Survivin study: an update of 'what is the next wave'? J Cell Physiol 2006, 208: 476–486.
- 7 Altieri DC. Survivin, versatile modulation of cell division and apoptosis in cancer. Oncogene 2003, 22: 8581–8589.
- 8 Miyachi K, Sasaki K, Onodera S, Taguchi T, Nagamachi M, Kaneko H and Sunagawa M. Correlation between survivin mRNA expression and lymph node metastasis in gastric cancer. Gastric Cancer 2003, 6: 217–224.
- 9 Rohayem J, Diestelkoetter P, Weigle B, Oehmichen A, Schmitz M, Mehlhorn J and Conrad K, *et al.* Antibody response to the turnor-associated

inhibitor of apoptosis protein survivin in cancer patients. Cancer Res 2000, 60: 1815–1817.

- 10 Takai N, Miyazaki T, Nishida M, Nasu K and Miyakawa I. Survivin expression correlates with clinical stage, histological grade, invasive behavior and survival rate in endometrial carcinoma. Cancer Lett 2002, 184: 105–116.
- 11 Kenney DM, Geschwindt RD, Kary MR, Linic JM, Sardesai NY and Li ZQ. Detection of newly diagnosed bladder cancer, bladder cancer recurrence and bladder cancer in patients with hematuria using quantitative rt-PCR of urinary survivin. Tumour Biol 2007, 28: 57–62.
- 12 Kishi H, Igawa M, Kikuno N, Yoshino T, Urakami S and Shiina H. Expression of survivin gene in prostate cancer: correlation with clinicopathological characteristics, proliferative activity and apoptosis. J Urol 2004, 171: 1855–1860.
- 13 Shariat SF, Lotan Y, Saboorian H, Khoddami SM, Roehrborn CG, Slawin KM and Ashfaq R. Survivin expression is associated with features of biologically aggressive prostate carcinoma. Cancer 2004, 100: 751–757.
- 14 Yang D, Welm A and Bishop JM. Cell division and cell survival in the absence of survivin. Proc Natl Acad Sci USA 2004, 101: 15100-15105.
- 15 Reed JC. The survivin saga goes in vivo. J Clin Invest 2001, 108: 965–969.
- 16 Altieri DC. Validating survivin as a cancer therapeutic target. Nat Rev Cancer 2003, 3: 46–54.
- 17 Zaffaroni N, Pennati M and Daidone MG. Survivin as a target for new anticancer interventions. J Cell Mol Med 2005, 9: 360–372.
- 18 Fire A, Xu S, Montgomery MK, Kostas SA, Driver SE and Mello CC. Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. Nature 1998, 391: 806–811.
- 19 Zamore PD. RNA interference: listening to the sound of silence. Nat Struct Biol 2001, 8: 746–750.
- 20 Cheng SQ, Wang WL, Yan W, Li QL, Wang L and Wang WY. Knockdown of survivin gene expression by RNAi induces apoptosis in human hepatocellular carcinoma cell line SMMC-7721. World J Gastroenterol 2005, 11: 756–759.
- 21 Sui G, Soohoo C, Affar el B, Gay F, Shi Y, Forrester WC and Shi Y. A DNA vector-based RNAi technology to suppress gene expression in mammalian cells. Proc Natl Acad Sci USA 2002, 99: 5515–5520.
- 22 Zhang M, Latham DE, Delaney MA and Chakravarti A. Survivin mediates resistance to antiandrogen therapy in prostate cancer. Oncogene 2005, 24: 2474–2482.
- 23 Shen JJ, Hao XK, Liu JY, Su MQ, Miao YY and Zhang Q. Inhibition of survivin gene expression in PC-3 cells by RNAi [Article in Chinese]. Zhonghua Nan Ke Xue 2006, 12: 890–895.
- 24 Xiao D, Wang K, Zhou J, Cao H, Deng Z, Hu Y and Qu X, et al. Inhibition of fibroblast growth factor 2-induced apoptosis involves survivin expression, protein kinase C α activation and subcellular translocation of Smac in human small cell lung cancer cells. Acta Biochim Biophys Sin (Shanghai) 2008, 40: 297–303.
- 25 Fukuda S and Pelus LM. Survivin, a cancer target with an emerging role in normal adult tissues. Mol Cancer Ther 2006, 5: 1087–1098.
- 26 Gong L, Jin X, Li Q, Liu J and An L. Heavy ion beams induce survivin expression in human hepatoma SMMC-7721 cells more effectively than X-rays. Acta Biochim Biophys Sin (Shanghai) 2007, 39: 575–582.
- 27 McEleny KR, Watson RW, Coffey RN, O'Neill AJ and Fitzpatrick JM. Inhibitors of apoptosis proteins in prostate cancer cell lines. Prostate 2002, 51: 133–140.

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- 28 Krajewska M, Krajewski S, Banares S, Huang X, Turner B, Bubendorf L and Kallioniemi OP, *et al.* Elevated expression of inhibitor of apoptosis proteins in prostate cancer. Clin Cancer Res 2003, 9: 4914–4925.
- 29 Fei Q, Zhang H, Fu L, Dai X, Gao B, Ni M and Ge C, *et al.* Experimental cancer gene therapy by multiple anti-survivin hammerhead ribozymes. Acta Biochim Biophys Sin (Shanghai) 2008, 40: 466–477.
- 30 Uchida H, Tanaka T, Sasaki K, Kato K, Dehari H, Ito Y and Kobune M, et al. Adenovirus-mediated transfer of siRNA against survivin induced

apoptosis and attenuated tumor cell growth *in vitro* and *in vivo*. Mol Ther 2004, 10: 162–171.

- 31 Li QX, Zhao J, Liu JY, Jia LT, Huang HY, Xu YM and Zhang Y, et al. Survivin stable knockdown by siRNA inhibits tumor cell growth and angiogenesis in breast and cervical cancers. Cancer Biol Ther 2006, 5: 860–866.
- 32 Roca H, Varsos Z and Pienta KJ. CCL2 protects prostate cancer PC3 cells from autophagic death via phosphatidylinositol 3-kinase/AKT-dependent survivin up-regulation. J Biol Chem 2008, 283: 25057–25073.