International Scholars Journals

International Journal of Diseases and Disorders ISSN 2329-9835 Vol. 3 (3), pp. 001-007, March, 2015. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Effect of shRNA-mediated survivin gene silencing on the biological behavior of human bladder transitional cell carcinoma T24 cells

Yunfeng He[1], Hu Du[1], Xiaohou Wu[1]*, Wei Tang[1], Chuan Liu[2], Zhikang Yin[1], Yao Zhang[1], Chunli Luo[3], and Jiabing Li[4]

[1]Department of Urology, The First Affiliated Hospital, Chongqing Medical University, Chongqing 400016, China.
[2]Department of Urology, The Second Affiliated Hospital, Chongqing Medical University, Chongqing 400016, China.
[3]Faculty of Laboratory Medicine, Chongqing Medical University, Chongqing 400016, China.
[4]The Third hospital of mianyang, No. 190 jiannian Road, mianyang; 621000 China.

Accepted 13 September, 2014

To construct the expression vector of the survivin-specific short hairpin RNA (shRNA) and to investigate its effect on the biological behavior of human bladder transitional cell carcinoma T24 cells. Survivin-specific short oligonucleotides were designed, synthesized, and cloned into the PTZU6+1 vector, resulting in a survivin-specific shRNA expression construct, which was then transfected into T24 cells. The mRNA and protein levels of survivin were determined by semi-quantitative reverse transcription polymerase chain reaction (RT-PCR) and Western blot analysis, respectively. Tumor cell growth, movement and neoplasm invasiveness were detected by a cell growth curve, a random motion experiment, and a Matrigel transmembrane assay. Target DNA fragments containing psurvivin-shRNA vectors was verified by *Eco*R I / *Hin*d III double digestion and sequencing and successfully transfected into T24 cells. Transfection of psurvivin-shRNA cells significantly inhibited mRNA levels by 61.73% and protein expression by 73.37%. The ability of cell invasion and movement was significantly decreased. The inhibition rates of tumor cell growth were 59.13% on day 3 and 83.86% on day 5. A survivin-specific shRNA expression vector was constructed based on the PTZU6 +1 vector and was transfected into T24 cells, which resulted in the inhibition of T24 cell proliferation and movement.

Key words: Survivin, short hairpin RNA, Neoplasm invasiveness, bladder neoplasms.

INTRODUCTION

The survivin gene, an apoptosis suppressor gene, was discovered in 1997 when Ambrosini et al. (1997) used the effector cell proteasereceptor-1 (epr-1) to screen against the human cDNA library. The human survivin gene is located on chromosome 17q25 with a length of 75-130kb, is encoded by 1.9 kb mRNA, and includes 3 introns and 4 exons. The 2560-2920 bp GC-rich region upstream of exon 1 is a non-TATA promoter. The survivin protein contains 142 amino acids with a molecular weight of 16.5 KDa (Ambrosini et al., 1997; Adida et al., 1998). As with other members of the inhibitors of apoptosis (IAP) family, surviving has a baculovirus IAP Repeat (BIR) structure that is essential for the anti-apoptotic function of survivin. However, unlike other IAP family members, survivin contains only one BIR structure and one α-helix structure at the carboxyl terminal, and the carboxyl terminal does not have a ring finger structure (Ambrosini et al., 1998; Tamm et al., 1998). Furthermore, it forms dimers that are dormant in crystal structures.

As survivin is a non-secreted protein, it would be detected in body fluids only if the malignant cells shed into body fluids and release intracellular survivin. This assumption was first confirmed in bladder cancer by Smith et al. (2001). Their results show that survivin expression could be detected in the urine of patients with new or recurrent bladder cancer. The sensitivity and specificity are 100 and 95%, respectively. Patients with

*Corresponding author. E-mail: wuxiaohou80@hotmail.com. Tel: 86-23-89011122. Fax: 86-23-89011122.

positive expression have high recurrence rates, indicating a poor prognosis. However, survivin was negative in the urine of healthy volunteers, patients with non-neoplastic urological diseases, and patients with non-urological neoplastic diseases. Kappler et al. (2004) reported the application of small interfering RNA (siRNA) in reducing the expression of survivin in five sarcoma cell lines with either wild type or different mutations. The results suggest that survivin-targeted siRNA can lead to specific apoptosis of tumor cells, regardless of the expression of wild-type p53 in sarcoma cells. As a result, survivin is expected to be the new hot spot bladder neoplasm research topic, with potential for the diagnosis and treatment of this type of cancer. In this study, using the transcription vector pTZU6 +1, a small hairpin RNA (shRNA) expression vector was constructed. This construct was applied to the study of endogenous survivin gene expression in T24 bladder transitional cell carcinoma and to the study of its association with the biological School behavior and metastatic potential of bladder transitional cell carcinoma.

MATERIALS AND METHODS

Cell culture

Bladder transitional cell carcinoma cell line T24 (courtesy of the fundamental Institute, Chongqing Medical University) was cultured in regular RPMI1640 culture medium containing 10% calf serum medium at 37°C in the presence of 5% CO_2 saturation. The growth of the cell culture was observed regularly. After every 2 to 3 days, the subculture was subjected to 0.25% trypsin digestion.

Survivin siRNA sequence selection and construction of a eukaryotic expression vector

The pTZU6 +1 expression plasmid was a gift from the hepatitis Institute of Chongqing Medical University. According to shRNA design principles and the survivin coding sequence, 19-21nt of the DNA oligos were designed such that they were spanned by a 4 or 8 nt insertion sequence (TTCG or TTAGTACT or GAGTACTG) with no homology to the targeted gene, and the sequence specificity was confirmed by BLAST analysis. In this study, both the survivin coding sequence and the reverse complementary sequence were synthesised, named as Survivin-siRNA (encoding gene 191-211), as follows: 5'-TCGAG TGAGAACGAGCCAGACTTGTTAGTACTCAAGTCTGGCTCGTTC TCAGTTTTT-3'(sense),5'-TAGAAAAACTGAGAACGAGCCAGACTTGGTACTAACAAGTCTG GCTCGTTCTCAGC-3' (antisense). XhoI or XbaI restriction sites were incorporated on the either end of the oligos for the cloning into the pTZU +1 vector.

Screening and verification of the pshRNA-Survivin expression plasmid

Annealed DNA fragments were purified using a DNA purification kit and ligated with a Sal I/Sal I digested pTZU6 +1 transcription vector with T4 DNA ligase. The ligation reaction proceeded at 4°C overnight, resulting in the recombinant plasmid as designed. A total of 7 µl of ligation mixture was used in the transformation of *Escherichia coli* JM109. Colonies were screened on a plate containing Amp. Plasmid DNA was prepared by mini prep, digested with Sal I, EcoR I, or Hind III and electrophoresized on 1% agarose gel with the pTZU6+1 vector digested with the same enzyme as controls. The positive recombinant plasmid was further confirmed by sequencing and named psurvivin-shRNA.

Cell transfection

Transfection was performed according to the manual of the Invitrogen transfection kit. Lipofectamine TM2000 (Invitrogen) and plasmid at a ratio of 1:2 were added to the T24 cells and incubated at 37°C with PTZU6+1 for 4-6 h in RPMI-1640 medium containing serum. Incubation with PTZU6+1 at 37 was continued for another 48 h before cell harvest. Experimental groups included the non-transfected group, the control group and the pshRNA-survivin group transfected with PBS, the negative control plasmid psurvivin-cRNA, and the psurvivin-shRNA plasmid, respectively.

Measurement of the T24 cell growth curve

After transfection and screening, target cells (1×10^6 cells) were synchronized by inoculation into serum-free DMEM medium containing 10 ug/ml of insulin in 25 cm^2 culture dishes for 24 h. Cell culture was washed three times with PBS (PH7.4) and was treated with 0.25% trypsin to obtain a single cell suspension at a concentration of 5×10^4/ml. A total of 200 µl of cell suspension was inoculated into each well in 96-well plates and incubated in 5% CO2 at 37 PTZU6 +1 for 18 h. Absorbance at 490 nm (OD490) of cells from the three groups was measured by the MTT method [3 -(4,5-dimethy1-2-thiazoly) -2,5-dipheny1-2H-tetrazolium bromide] over 24-120 h. At each time point, 20 ul of MTT solution was added into six wells and incubated at 37 PTZU6 +1 for 6 h. Then, 200 µl of supernatant was removed, and 200 µl of DMSO was added into each well. After soft shaking, the OD490 was measured by ELISA. In the cell growth curve, the time points were set as the abscissa and the absorbance as the vertical axis. To evaluate the in vitro cytotoxicity of psurvivin-shRNA to T24 cells, psurvivin-shRNA inhibition of T24 cells was calculated according to the following formula:

Tumor inhibition rate = (mean OD490 of controls - OD490 of test) / mean OD490 of controls × 100%.

Boyden invasion assay

Matrigel (BD Biosciences, US) was diluted to 5 mg/ml in serum-free DMEM medium. A polycarbonate porous membrane (diameter = 13 mm, pore size = 8 µm) was inserted between the upper and lower chamber of the transwell. Artificial Matrigel was then spread evenly over the membrane at 50 µg / hole. DMEM medium containing 5 µg/ml of fibronectin was added to the lower chamber of the transwell. Cells were resuspended in DMEM medium containing 1% FBS to 10^5 cells/ml. Then, 500 µl of cell suspension was transferred onto the Matrigel, incubated in 5% CO_2 at 37 PTZU6 +1 for 12 h, and then transfered into a transwell followed by formaldehyde fixing and HE staining. Cells without invasion and Matrigel were removed from the upper chamber with a sterile cotton swab. Three parallel samples were set as a group. The number of cells with invasion was observed from five views for each membrane and was counted under a light microscope at a 400x magnification. The mean value was calculated.

Random movement experiment

About 1×10^6 tumor cells were inoculated into three Petri dishes

Fig 1 Screening and identification of the recombinant plasmid psurvivin-shRNA

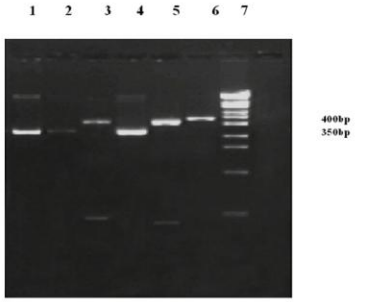

Figure 1: Identification of pshRNA-Survivin2 with restriction-endonuclease digestion

1.Plasmid Clone 3
2.Plasmid Clone 3 digested with Sal Ⅰ
3.Plasmid Clone 3 digested with HindⅢ and EcoR Ⅰ
4.Plasmid *pTZU*6+1
5.Plasmid *pTZU*6+1 digested with HindⅢ and EcoR Ⅰ
6.Plasmid *pTZU*6+1 digested with Sal Ⅰ
7.DNA Marker

Figure 1. Screening and identification of the recombinant plasmid psurvivin-shRNA.1. Plasmid Clone 3; 2. Plasmid Clone 3 digested with Sal I; 3. Plasmid Clone 3 digested with HindIII and EcoR I; 4. Plasmid *pTZU*6+1; 5. Plasmid *pTZU*6+1 digested with Hind III and EcoR I; 6. Plasmid *pTZU*6+1 digested with Sal I; 7. DNA Marker.

and grown to saturation density. A strip on the dish was scraped, and the cells scraped were washed away with PBS. The cell culture was incubated for 48 more hours before inverted microscopy analysis.

Chemotaxis test

The procedure for the in vitro chemotaxis test is the same as that for the in vitro invasion assay, except that the membrane of the invasion chamber is not covered with artificial matrigel. The cells were inoculated into the upper chamber of the transwell (2×10^5 cells per chamber), and 1 ml of conditional medium containing FN was introduced into the bottom chamber. After incubation, the membrane was removed, followed by methanol fixing and HE staining. Cells that did not move through the membrane were wiped away with a cotton swab. The slide was sealed. Photography and cell count were performed under a high magnification microscope. Each group included three parallel wells.

Changes of survivin mRNA measured by Semi-quantitative RT-PCR

RT-PCR was performed according to the kit instructions. An amount of 2μg of total RNA was used in the RT-PCR, with a kit with reference to the operating instructions. The survivin gene primers used were 5'-ATAGTCGACATGGGTGCCCCGACGTTG -3 '(upstream primer) and 5'-CTCGGATCCCAATCCATGGCAGCCAGCT-3 '(downstream primer). The size of the amplification product was 421 bp. The reaction conditions were as follows: 50 PTZU6 +1 for 30 min for HotStarTaq enzyme activation, 95 PTZU6 +1 for 15 min, and then the following PCR cycles: denaturation at 94 PTZU6 +1 for 45 Sec, annealing at 60 PTZU6 +1 for 45 s, elongation at 72 PTZU6 +1 for 1 min (Total 30 cycles), extension at 72 PTZU6 +1 for 10 min.

Then, 10 μl of PCR product was subjected to 1% agarose gel electrophoresis. A 420 bp band represented a positive result. The band was quantified by a BIO-RAD image analyzer.

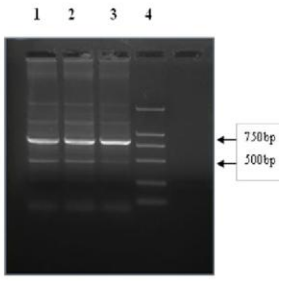

Figure 2. Semi-quantitative RT-PCR analysis of survivin and GAPDH. 1. PBS group; 2. pSurvivin-cRNA group; 3. psurvivin-shRNA group; 4. MARKER2000.

Detection of survivin protein expression by Western blot

T24 cells were lysed in a lysis buffer (0.5 mol / L Tris-Cl PH 6.8,10% SDS, 1 M DTT) and were denatured at 100 PTZU6 +1 for 5 - 10 min followed by immediate cooling in ice water and centri-fugation at12000 r/min for 10 min at 4 PTZU6 +1. Protein samples were subject to 10% SDS-PAGE and transferred to a nitrocellulose membrane. The membrane was incubated with an anti-survivin primary antibody (1:1000, Santa Cruz) overnight at 4 PTZU6 +1 followed by an HRP-conjugated goat anti-rabbit IgG secondary antibody (1:2000, Beijing Zhongshan Biotechnology Company) at 37 PTZU6 +1 for 1 h. The reaction solution was discarded, and the membrane was incubated in a chemiluminescence reagent for 5 min. The result was observed and photographed using a Syngene Bio Imaging detection system. The bands were analyzed with a BIO-RAD image analyzer.

Statistical analysis

The data were analyzed and tested using SPSS 10.0 software. Variance analysis between groups was performed.

RESULTS

Plasmid construction

Plasmid psurvivin-shRNA could be digested with Hind III and EcoR I, but not with Sal I, indicating the successful construction of the plasmid (Figure 1).

Detection of survivin mRNA expression levels by semi-quantitative RT-PCR

Agarose gel electrophoresis results (Figure 2) showed that amplified bands of endogenous GAPDH genes from

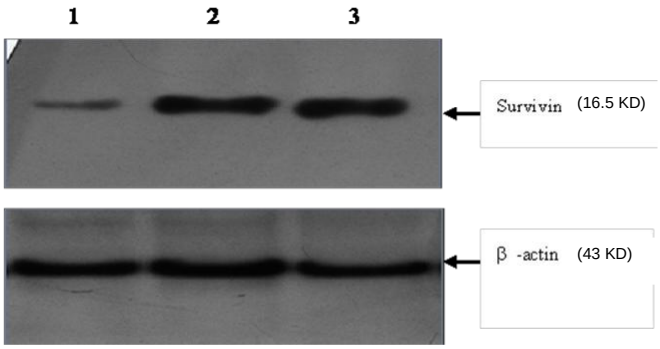

Figure 3. Detection of survivin expression in transfected cells by Western blot analysis.
1. pshRNA-survivin group; 2. pSurvivin-cRNA group; 3. PBS group.

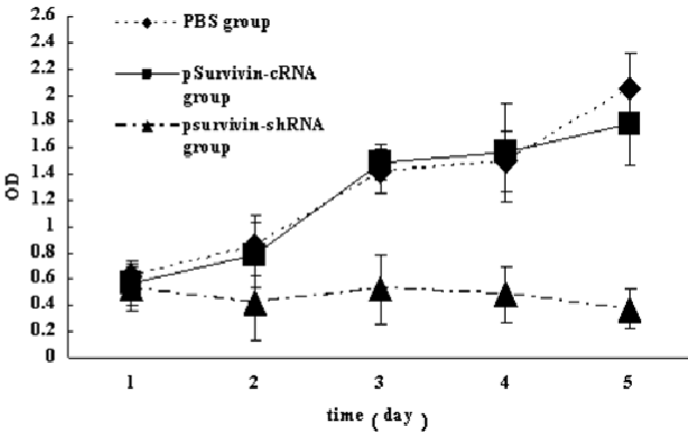

Figure 4. T24 cell growth curve.

3 groups were similar in brightness intensity, indicating that the same amount of added templates were present in RT-PCR. The bands of the survivin gene amplification from each group were obviously different. Both the pSurvivin-cRNA group and the PBS group had strong survivin PCR amplification, while the psurvivin-shRNA group was significantly decreased in the survivin PCR amplification. The significant difference between the psurvivin-shRNA group and the control groups suggested that the recombinant plasmid psurvivin-shRNA was able to silence the survivin gene at them RNA level.

Quantitative analysis using a BIO-RAD electrophoresis image analyzer indicated that the inhibition rates of the pSurvivin-cRNA group and the psurvivin-shRNA were 8.48 and 61.73%, respectively. The RT-PCR band from the non-transfection group was set as a standard (intensity 100). As the bands of the endogenous GAPDH gene were quite similar in intensity, the bands of the survivin gene from each group were compared.

Detection of survivin protein expression by Western blot analysis

After siRNA transfection, survivin protein expression was detected by Western blot analysis. The results showed that survivin protein levels in the PBS group and the pSurvivin-cRNA group were significantly stronger than the corresponding levels in the experimental group with pshRNA-survivin transfection, indicating that pshRNA-survivin could effectively inhibit the expression of survivin protein. Quantative analysis (Figure 3) by a BIO-RAD image analyzer showed that, compared to PBS, the inhibition rate of pshRNA-survivin was 72.27% at the protein level. The difference was statistically significant (P <0.01).

T24 cell growth curve

There was no obvious difference between the PBS group and the pSurvivin-cRNA in cell growth, whereas the cell growth of the pshRNA-survivin group was obviously inhibited, suggesting that pshRNA-survivin could inhibit the growth of T24 cells (Figure 4). An MTT test showed that tumor cell growth inhibition rates at 3 and 5 days were 59.13 and 83.86%, respectively.

Boyden invasion assay

The number of transmembrane cells in the pshRNA-survivin group was 15.34 ± 1.85, while the corresponding number in the PBS group and the pSurvivin-cRNA group were 27.62 ± 2.06 and 26.07 ± 1.73, respectively. The difference was determined to be significant (P <0.01) (Figure 5).

Movement experiment

Random movement experiment

Compared to the PBS group and the pSurvivin-cRNA group, the random movement of cells in the pshRNA-survivin group revealed a large empty area (Figure 6).

Chemotaxis experiment

The number of transmembrane cells in the pshRNA-survivin group was 41.32 ± 3.47, while the number in the PBS group and the pSurvivin-cRNA group were 62.84 ± 4.97 and 64.15 ± 6.71, respectively. The difference was determined to be significant at P <0.01 (Figures 7 and 8).

DISCUSSION

Survivin was discovered by Altieri in 1997 as a new

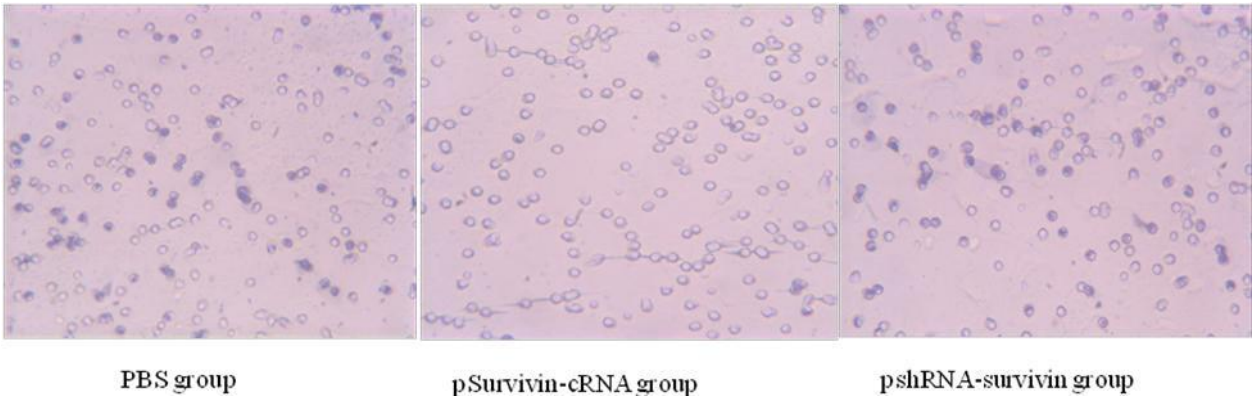

Figure 5. Invasion assay.

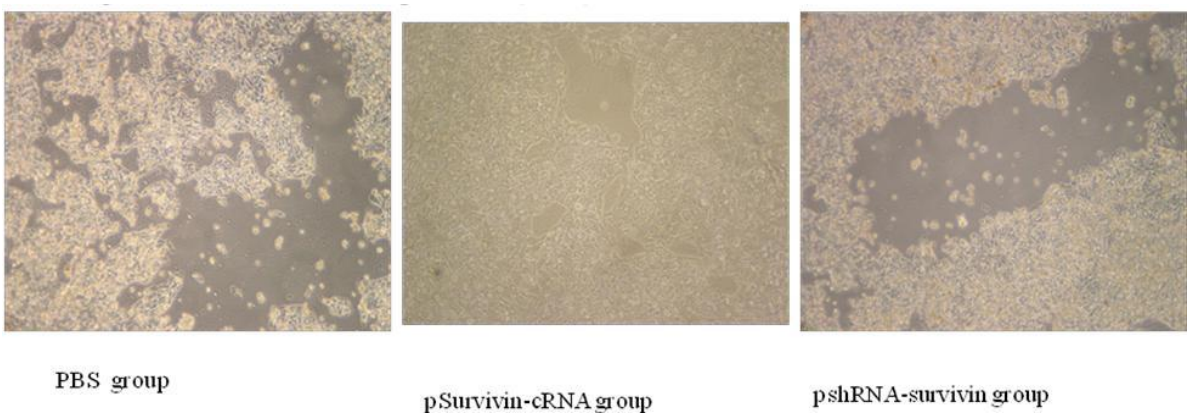

Figure 6. Random movement experiment (× 100).

inhibitor of apoptosis proteins (IAPs) (Ambrosini et al., 1997; Adida et al., 1998; Ambrosini et al., 1998; Tamm et al., 1998). Because of its unique biological structure, tissue distribution and apparent specific anti-apoptotic effect, survivin has been a topic of intense research. Apoptosis has two major pathways, the receptor apoptotic pathway and the mitochondrial pathway. In both pathways, the final terminal effectors are caspase-3 and caspase-7. In vitro experiments confirmed that survivin inhibits apoptosis by directly or indirectly affecting the function of caspase. Survivin could directly inhibit the activities of caspase-3 and caspase-7 to block the apoptosis process. Survivin can also indirectly inhibit caspase-3 through P21. The formation of the survivin-CDK4 (cell cycle modulation factor) complex leads to the release of P21 from the CDK complex (Szentmáry et al., 2010). Released P21 interacts with caspase-3 to inhibit its activity. P21 could also translocate to the mitochondria and form complexes with procaspase-3, resulting in the inhibition of caspase-3 activity and the blocking of cytochrome c from the mitochondria. Thus, apoptosis was inhibited. Study of positioning in the process of cell division has shown that, together with INCENP (inner centromere protein) and Aurora-B, survivin plays an important role in cell cycle regulation and mitosis. Survivin can also inhibit apoptosis by activating NF-κB, which is mediated by the tumor necrosis factor receptor. Studies also show that survivin inhibits apoptosis induced by IL-3, fas, bax, or TNF-α (Kanwar et al., 2011; Skagias et al., 2009; Zhang et al., 2009; Lin et al., 2010; Uren et al., 2000; Deveraux et al., 1999).

In this study, the transcription vector pTZU6+1 was used to construct recombinant plasmid pshRNA-survivin for the production of siRNA against survivin in mammalian cells. The results showed that both transcription and protein levels of survivin were inhibited in bladder transitional cell T24 carcinoma cell lines that were transfected with pshRNA-survivin. RT-PCR indicated that survivin mRNA was inhibited by 61.73%. Western blot analysis revealed that in T24 tumor cells, pshRNA-survivin could effectively inhibit the expression of survivin with an inhibition rate of 73% or above as well as with

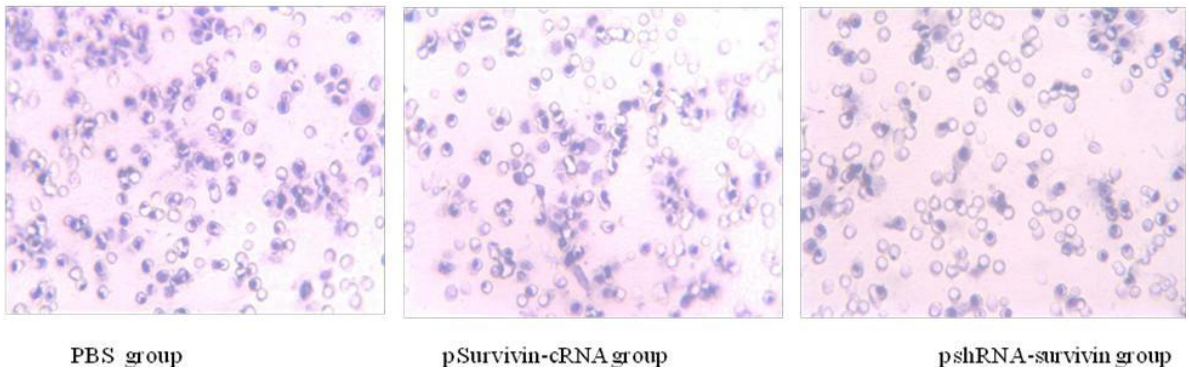

Figure 7. Chemotaxis experiment (HE × 400).

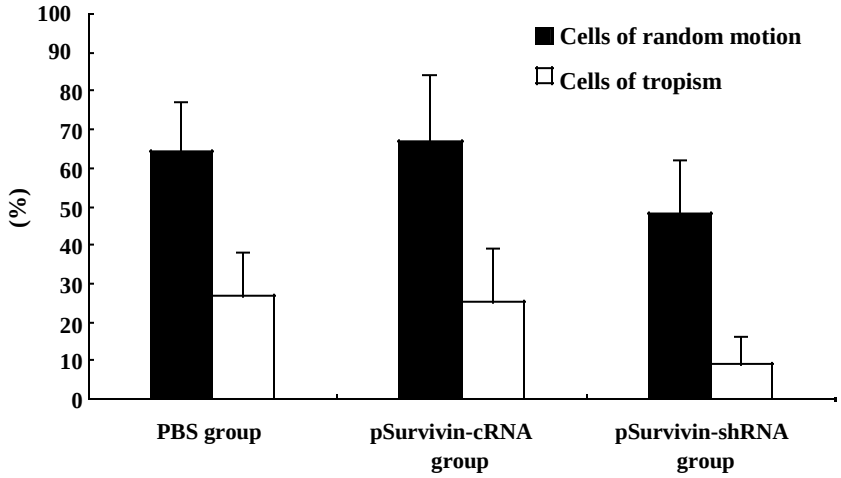

Figure 8. Effect of pshRNA-survivin transfection on T24 cell motility and invasion.

high specificity. Here, we constructed pshRNA-survivin that was able to effectively and specifically downregulate the transcription and translation of survivin in T24 cells, providing a useful platform for further experiments.

Established by Albini, a Boyden invasion assay was also known as a fast in vitro invasion assay. Kleiman et al. (1986) believed that, theoretically, the thicker the film and the longer the invasion time, the easier it is to determine the difference of invasion ability between different tumors. However, chemotaxis may interfere with the assay over time, and the stability of artificial Matrigel will be affected as well. Therefore, 50 μg of artificial Matrigel were used in this study to achieve the desired coverage and the ideal thickness, and the membrane was treated with HE staining after invasion for 12 h. Random views from different regions were selected for cell counting, but errors could still exist (Hagglund et al., 2009; Gohla et al., 1996; Garbisa et al., 1980).

The results of this study showed the following: 1, cell invasiveness is positively correlated with movement ability; 2, the invasiveness of cancer cells could be deferred indirectly from movement ability and the cancer cell growth curve; 3, cell invasiveness and movement ability significantly decreased in the pshRNA-survivin group; and 4, on the 3rd day and 5th day, tumor cell growth inhibition rates were 59.13 and 83.86%, respectively. The results showed that the silencing of the survivin gene could lead to a significant decrease of T24 cell invasiveness and movement ability. The mechanisms for this are still not clear. Whether E-cadherin and β-catenin-Tcf/Lef pathways are involved will be further investigated.

Conclusions

Taken together, in T24 cells of bladder transitional cell carcinomas, the specific silencing of the survivin gene could lead to a significant decrease in T24 cell invasiveness and movement ability.

ACKNOWLEDGEMENTS

This study was supported by research grants from Research Development Foundation of Health Bureau of Chongqing (No. 2011-2-103), and was supported by the Foundation of National Natural Science Foundation of China (Grant No. 81000002).

REFERENCES

Adida C, Crotty PL, McGrath J, Berrebi D, Diebold J, Altieri DC (1998). Developmentally regulated expression of the novel cancer anti-apoptosis gene survivin in human and mouse differentiation. Am. J. Pathol., 152(1):43-49.

Ambrosini G, Adida C, Altieri DC (1997). A novel anti-apoptosis gene, survivin, expressed in cancer and lymphoma. Nat. Med., 3(8):917-921.

Ambrosini G, Adida C, Sirugo G, Altieri DC (1998). Induction of apoptosis and inhibition of cell proliferation by survivin gene targeting. J. Biol. Chem., 273(18): 11177-11182.

Deveraux QL, Reed JC (1999). IAP family proteins--suppressors of apoptosis., Genes Dev., (3):239-252.

Garbisa S, Kniska K, Tryggvason K, Foltz C, Liotta LA(1980). Quantitation of basement membrane collagen degradation by living tumor cells in vitro., Cancer Lett., 9(4):359-366.

Gohla A, Eckert K, Maurer HR (1996). A rapid and sensitive fluorometric screening assay using YO-PRO-1 to quantify tumour cell invasion through Matrigel., Clin. Exp. Metastasis, 14(5):451-458.

Hagglund S, Hoppe A, Aubyn D, Cavanna T, Jordan P, Zicha D (2009). Novel shear flow assay provides evidence for non-linear modulation of cancer invasion., Front Biosci., 14:3085-3093.

Kanwar JR, Kamalapuram SK, Kanwar RK (2011). Targeting survivin in cancer: the cell-signalling perspective., Drug Discov. Today, 16(11-12):485-4, 2011.

Kappler JA, Starr CJ, Chan DK, Kollmar R, Hudspeth AJ (2004). A nonsense mutation in the gene encoding a zebrafish myosin VI isoform causes defects in hair-cell mechanotransduction. Proc. Natl. Acad. Sci. USA. 101(35):13056-13061

Kleinman HK, McGarvey ML, Hassell JR, Star VL, Cannon FB, Laurie GW, Martin GR (1986). Basement membrane complexes with biological activity. Biochem., 25(2):312-318.

Lin J, Guan Z, Wang C, Feng L, Zheng Y, Caicedo E, Bearth E, Peng JR, Gaffney P, Ondrey FG (2010). Inhibitor of differentiation 1 contributes to head and neck squamous cell carcinoma survival via the NF-kappaB/survivin and phosphoinositide 3-kinase/Akt signaling pathways., Clin. Cancer Res., 16(1):77-87.

Skagias L, Politi E, Karameris A, Sambaziotis D, Archondakis A, Ntinis A, Moreas I, Vasou O, Koutselini H, Patsouris E (2009). Survivin expression as a strong indicator of recurrence in urothelial bladder cancer Predictive value of nuclear versus cytoplasmic staining., Anticancer Res., (10):4163-4167.

Smith SD, Wheeler MA, Plescia J, Colberg JW, Weiss RM, Altieri DC (2001). Urine detection of survivin and diagnosis of bladder cancer., JAMA., 285(3):324-32,.

Szentmáry N, Stündl A, Szende B, Süveges I (2010). P21, p27, bax, cathepsin and survivin pathways in macular dystrophy corneas., Histol. Histopathol., 25(3):287-290.

Tamm I, Wang Y, Sausville E, Scudiero DA, Vigna N, Oltersdorf T, Reed JC (1998). IAP-family protein survivin inhibits caspase activity and apoptosis induced by Fas (CD95), Bax, caspases, and anticancer drugs., Cancer Res., 58(23):5315-5320.

Uren AG, Wong L, Pakusch M, Fowler KJ, Burrows FJ, Vaux DL, Choo KH (2000). Survivin and the inner centromere protein INCENP show similar cell-cycle localization and gene knockout phenotype., Curr. Biol., (21):1319-1328.

Zhang W, Tong Q, Wu Q, Li S, Wang X, Wang Q (2009). Upregulated p27kip1 can downregulate survivin expression and inhibit telomerase activity in gastric carcinoma cells., Cancer. Invest., (9):898-900.