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Original Article

Synergistic antitumor effect of TRAIL and IL-24 with complete eradication of hepatoma in the CTGVT-DG strategy

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The ZD55-tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) and ZD55-interleukin (IL)-24 were constructed by inserting *TRAIL* or *IL-24* gene separately into the oncolytic adenovirus named ZD55 (with adenovirus E1B-55kD deletion). The resulting ZD55-TRAIL and ZD55-IL-24 were used in combination to treat xenograft tumors in nude mice model. The results showed that it can not only completely eliminate BEL7404 hepatoma xenograft but also have excellent antitumor effect against gaster, lung, prostate, and breast carcinomas. It was also found that ZD55-TRAIL could not only suppress the tumor growth promoting effect by ZD55-IL-24 at lower dosage, but also substantially reduce the cancer cell viability in their combined use. This is because ZD55-IL-24 and ZD55-TRAIL could mutually enhance each other's antitumor effect greatly. All these findings conspicuously showed the synergistic antitumor effect of TRAIL and IL-24, which is also the reason for the antitumor effect by the combined use of TRAIL and IL-24 *in vitro* and also *in vivo*.

Keywords TRAIL; Mda-7/IL-24; cancer-targeting dual gene-viro-therapy (CTGVT-DG); cancer therapy; apoptosis

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Introduction

Cancer has recently become the first leading cause of death in developed countries and in metropolitans of developing countries. According to the statistics of the WHO in 2008, there were 12.6 million cancer patients, among which 7.5 million died. In order to cure cancer patients, many therapeutical strategies have been carried out clinically including surgery, chemotherapy, radiation, and so forth. Our lab has developed a cancer-targeting gene-viro-therapy (CTGVT) strategy by combining the advantages of both gene therapy and oncolytic virus (OV) therapy, which was constructed by inserting an antitumor gene into an OV vector. The CTGVT (OV-gene) strategy was initiated by our lab in 2001 [1] and the details of this strategy were published in 2003 [2]. The CTGVT (gene armed oncolytic viral therapy or OV-gene) is the same in principle and methodology in their construction, and has much better antitumor effect than that of either gene therapy or oncolytic adenovirus therapy alone. The reason for the better antitumor effect of CTGVT is that the OV itself has antitumor effect and can be replicated specifically in tumor cell several hundred folds, meanwhile the inserted genes can also be replicated at the same magnitude. The CTGVT strategy (OV-gene) has been persistently studied for more than 10 years in this lab, and a series of articles has been published [references]. Currently CTGVT (OV-gene) has become a hot topic for antitumor research and therapy, which has been validated by a deal of Amgen Inc. which paid 1 billion USD to purchase an OV-gene product, OncoHSV-GM-CSF (the OV from herpes simplex virus harboring the granulocyte-macrophage-colony-stimulating factor gene) [3], also by a Nature paper of OncoPox-GM-CSF, which is the first virus that is administrated by intravenous injection and could target the metastasized tumor [4], and also by our OncoAd-IL-24, which has much better antitumor effect than that of Ad-IL-24 [5].

Furthermore, many modifications and improvements have been made for CTGVT. One of which is to combine two genes that may have compensative or synergistic effect. This strategy was named cancer-targeting dual gene-viro-therapyCTGVT-DG. The combination of ZD55-tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) and ZD55-IL-24 is a typical CTGVT-DG, which could completely eradicate colorectal cancer xenograft [6]. Other combinations of two genes which could completely eliminate xenograft tumors were also reported [7, 8].

Mda-7 (melanoma differentiation associated gene-7) belongs to the interleukin 10 (IL)-10-related family and

later was named IL-24. The over-expression of IL-24 could inhibit cancer cell growth and induce apoptosis in many kinds of cancer cell lines, including melanoma, breast, lung, cervix, gliomas, colon, pancreas, and prostate carcinomas, but not in many normal cells [9–16]. TRAIL, a member of the tumor necrosis factor super family 10, can induce apoptosis in many cancer cell lines through activating death receptors [17–23], and TRAIL has no toxicity, especially when it is inserted into OV [24]. *TRAIL* and *IL-24*, two conspicuous antitumor genes which are widely used in cancer therapy, have been cloned into ZD55 vector, respectively. When ZD55-TRAIL combined with ZD55-IL-24, an excellent antitumor effect on human colorectal carcinoma was obtained [6]. Besides, the CTGVT-DG triggered robust apoptosis of cancer cell in the liver, gaster, lung, and breast and inhibited these tumor growth more effectively than that of either ZD55-TRAIL or ZD55-IL-24 treatment alone (in this paper). In all these six cancer cell xenografts, the antitumor effect of combined treatment was remarkable, as indicated by the complete elimination of tumor masses of colorectal and liver carcinoma in nude mice model. Such an excellent antitumor effect has never been reported before. The important finding of this paper is the synergistic antitumor effect of ZD55-TRAIL and ZD55-IL-24, as the ZD55-IL-24 could induce the expression of TRAIL and the ZD55-TRAIL could up-regulate the antitumor effect of ZD55-IL-24, which has not been reported before either.

Materials and Methods

Cell lines and culture conditions

HEK293 (embryonic kidney containing the E1A region of Ad5) was obtained from Microbix Biosystem Inc. (Toronto, Ontario, Canada). BEL-7404 (human hepatocarcinoma), A549 (human lung carcinoma), SGC-7901 (human gastric carcinoma), Bcap-37 (human breast carcinoma), and DU-145 (human prostate carcinoma) were purchased from the Cell Bank of Type Culture Collection of Chinese Academy of Sciences (Shanghai, China). HEK293, BEL-7404, A549, SGC-7901, and SW620 cell lines were cultured in Dulbecco's modified essential medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum, 4 mM glutamine, 50 U/ml penicillin, and 50 μg/ml streptomycin Bcap-37.

Virus propagation, purification, and titration

Oncolytic adenovirus ZD55, ZD55-EGFP (ZD55 carrying enhanced green fluorescent protein), ZD55-TRAIL (ZD55 carrying TRAIL), and ZD55-IL-24 (ZD55 carrying IL-24) were constructed previously in our lab [2]. These viruses and ONYX-015 were propagated on HEK293 cells and purified by cesium chloride gradient ultracentrifugation. The multiplicity of infection (MOI) of the virus preparations was determined by a plaque assay on HEK293 cells.

Cell viability assay

The cells were plated on 96-well plate and infected with ONYX-015, ZD55-EGFP, ZD55-IL-24, and ZD55-TRAIL or the two latter's combination. The cells were treated at various MOIs or with different times (24, 48, 72, or 96 h). Then, 20 μl of 5 mg/ml MTT (Sigma, St Louis, USA) solution was added into each well. The cells were incubated at 37°C for 4 h and the medium was removed. HCl-isopropanol solution (0.04 M, 100 μl) was added to each well to solubilize the formazan crystals. The absorbance was read on a Bio-Rad Microplate Reader (Bio-Rad, Hercules, USA) at dual wavelength mode (595 and 655 nm).

Flow cytometric analysis

Both adherent and floating cells were collected at special time points after adenovirus infection. The cells were washed twice with phosphate buffered saline (PBS), fixed with 75% ethanol and stored at −20°C for more than 1 h. Then the cells were centrifuged, washed with PBS and digested with 100 ml RNase A (0.5 g/l) at 37°C for 30 min. Propidium iodide (PI) solution (100 mg/ml PI, 1% Triton X-100, 150 mM NaCl; 400 ml) was added and the cells were incubated at 37°C for 30 min in dark. The sub-G0/G1 cellular DNA content was measured to calculate the apoptosis ratio by using FACScan flow cytometer equipped with CELLQUEST and ModFIT LT for Mac V1.01 software (BD Biosciences, Franklin Lakes, USA).

Western blot analysis

The cells were harvested from the plates by trypsinization and resuspended in lysis buffer (62.5 mM Tris–HCl, pH 6.8, 2% sodium dodecyl sulfate, 10 mM glycerol, 1.55% dithiothreitol). The total protein concentrations were determined by the Bio-Rad protein assay system and western blot analysis was carried out using standard protocol. The primary antibodies used were mouse monoclonal anti-mda-7/IL-24 (GenHunter Corporation, Nashville, USA), anti-TRAIL (R&D Systems, Minneapolis, USA), anti-caspase-3, anti-caspase-8, anti-caspase-9, anti-Parp, anti-E1A, and anti-actin (Santa Cruz Biotechnology, Santa Cruz, USA). The membranes were detected by the appropriate secondary antibodies and revealed with an enhanced chemiluminescence system (Amersham Life Sciences Inc., Arlington Heights, USA).

Animal experiments

All male and female Balb/c nude mice (4 weeks old) used in these experiments were obtained from the Animal Research Committee of the Institute of Biochemistry and

Cell Biology, Chinese Academy of Sciences (Shanghai, China). Animal experiments were performed according to the Guide for the Care and Use of Laboratory Animals [25]. 2×10^6 cells in 150 μl DMEM (for BEL-7404, SGC-7901, A549, and Bcap-37) or 5×10^6 cells (for Du145) were infected into the right flank of Balb/c nude mice to establish xenograft tumor model. The tumor was monitored and measured weekly and the volume (V) was calculated using the formula $V = 0.5 \times \text{length} \times \text{width}^2$. When the tumors were 100–120 mm^3 in size, the mice were randomly divided into six groups and different viruses were injected every other day for four times with total amount of 2×10^9 PFU per mouse or PBS similarly.

Statistical analysis

Data were presented as the mean ± SD from three independent experiments. Student's t-test was used to calculate the statistical significance of the experimental results. For all experiments, statistical significance is indicated as follows: NS, non-significant; $^{**}P < 0.01$; $^{***}P < 0.001$.

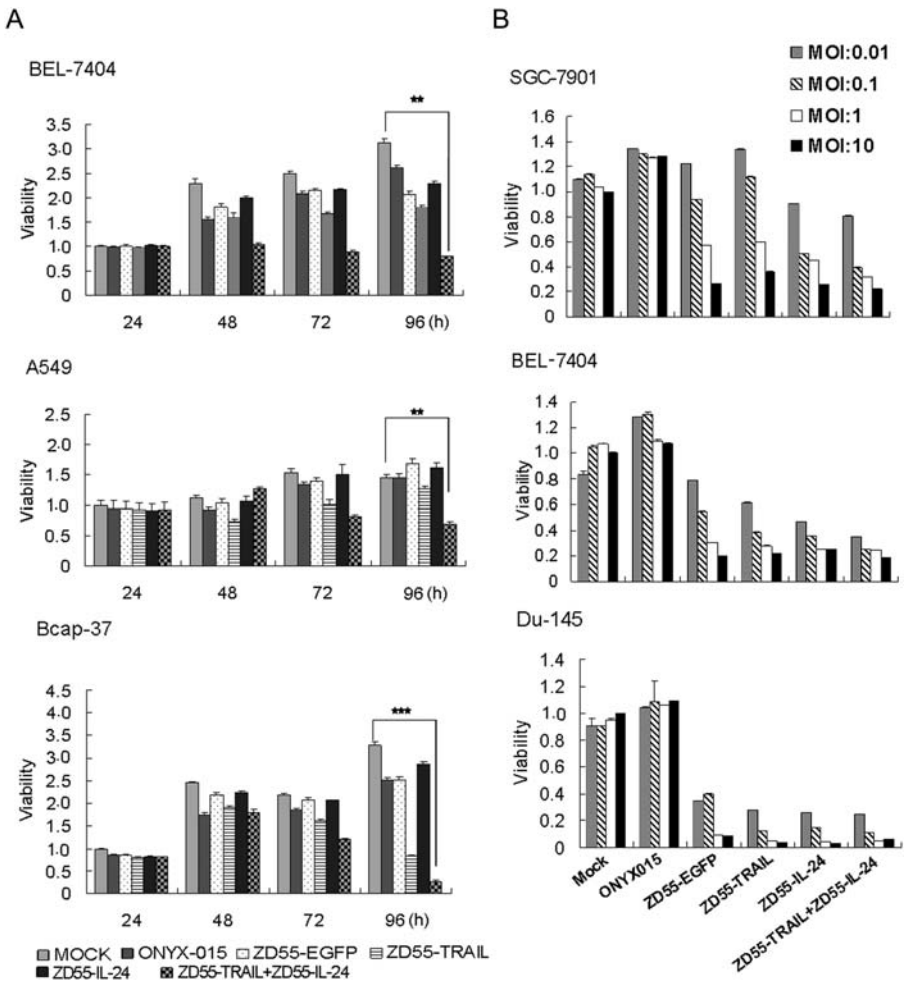


Figure 1 ZD55-IL-24 in lower dosage could promote cancer growth, and ZD55-TRAIL could suppress this effect of ZD55-IL-24 to reduce the cancer cell viability at different time, especially at 96 h treatment (A) BEL-7404, A549, and Bcap-37 cancer cell lines were cultured alone (Mock) or infected with ZD55-TRAIL and ZD55-IL-24 at lower dosage (1 MOI) separately or in combination, using ONYX-015 or ZD55-EGFP also as control. MTT assays were performed after 24–96 h post-injection; cell viability was increased in most separate treatment to promote the cancer growth. The most cancer-promoting effect group is ZD55-IL-24 and the least one is ZD55-TRAIL. Furthermore, ZD55-TRAIL could inhibit the cancer growth-promoting effect of ZD55-IL-24 and decrease the cancer cell viability, which is time dependent. This function of ZD55-TRAIL decreases strongly the viability when combined use of ZD55-TRAIL and ZD55-IL-24. The combined treatment of ZD55-TRAIL and ZD55-IL-24 results in an obvious decrease of viability ($n = 4$, $^{**}P < 0.01$, $^{***}P < 0.001$ which has occurred in the Bcap37 at 96 h). (B) BEL-7404 and SCG-7901 cell lines were infected with the ZD55-TRAIL and ZD55-IL-24 alone or in combination, at different MOIs (0.01, 0.1, 1, or 10). Cell viability was decreased at 48 h post-injection. It was found that TRAIL could be induced by 5-MOI ZD55-IL-24 in A549 cell line and more TRAIL were expressed when 10-MOI ZD55-IL-24 was used, indicating the up-regulation of TRAIL expression by ZD55-IL-24 treatment. In the BEL7404 case the combination of two CTGVT did not suppress much on the cancer viability because of the cancer-promoting effect of ZD55-IL-24.

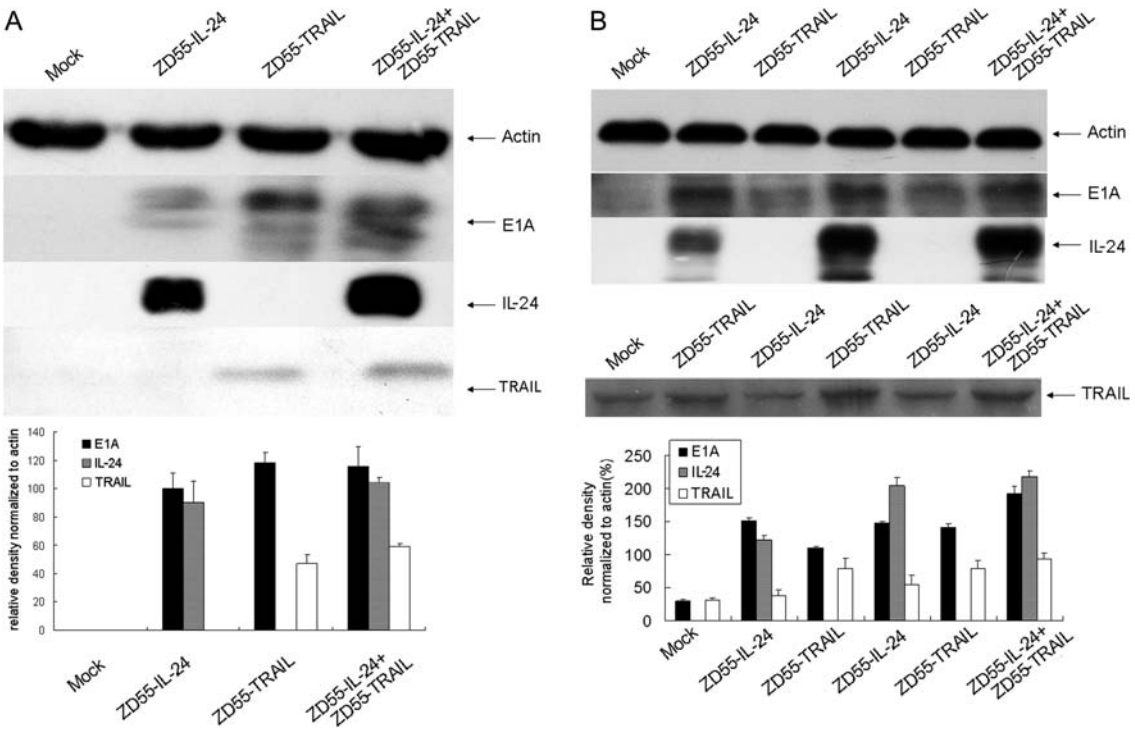


Figure 2 Induction of TRAIL expression by ZD55-TRAIL Different cancer cell lines BEL-7404 (A) and A549 (B) were treated at 5 MOIs or 10 MOIs of ZD55-TRAIL and ZD55-IL-24 separately or in combination. The cell lysate was collected after 48 h infection to detect E1A, IL-24, and TRAIL. The bands of E1A and IL-24 were diverging because of their isomers then their quantity were determined by densitometric analysis and showed in a column diagram. The E1A of adenovirus was not changed, that means no affect of TRAIL or IL-24 on adenovirus replication after different treatment. We focused on the up-regulation of TRAIL expression by ZD55-IL-24. The A549 cell line, treated with 5-MOI ZD55-IL-24 without ZD55-TRAIL, but there are TRAIL expression and more TRAIL were expressed when treated with 10-MOI ZD55-IL-24, showing the up-regulation of TRAIL expression by ZD55-IL-24.

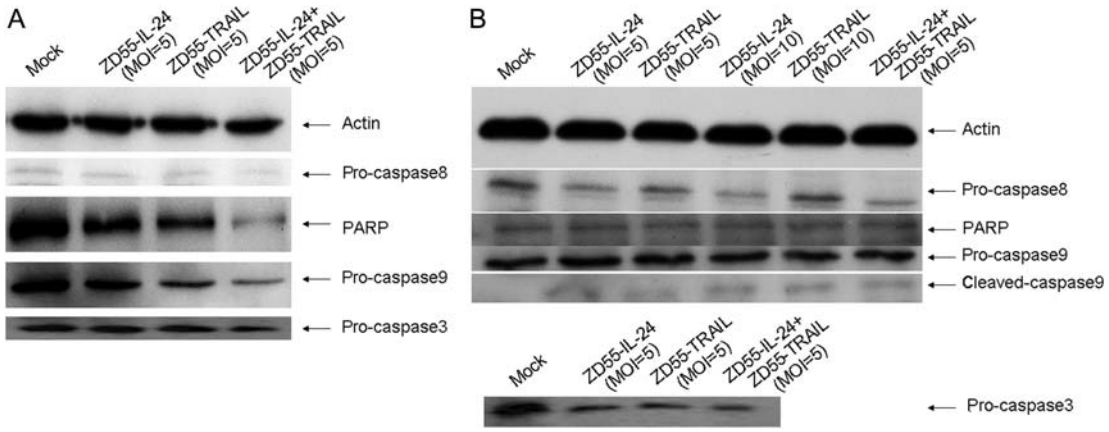


Figure 3 Antitumor effect of ZD55-TRAIL and ZD55-IL-24 or their combination by the induction of apoptosis mediated through caspase cascade SGC-7901 cell line (A) and A549 cell line (B) were treated with different CTGVT-viruses at five MOIs, collected all adherent and suspension cells by centrifugation after 48-h treatment. Western blot was conducted with the cell lysis. Pro-caspase-3, -8, -9, and PARP were decreased, indicating that the apoptosis was mediated through mitochondrial (intrinsic) and non-mitochondrial (extrinsic) pathway.

Results

ZD55-IL-24 in lower dosage could promote cancer growth, and ZD55-TRAIL could suppress this effect of ZD55-IL-24 to reduce finally the cancer cell viability

The cancer cell viability of ZD55-TRAIL and ZD55-IL-24 treatment separately or in combination in lower dosage (here 1 MOI) was detected with MTT assay at different time points [**Fig. 1(A)**], using ONYX-015 and ZD55-EGFP as controls. As shown in **Fig. 1(A)**, all the ONYX-015, ZD55-EGFP, ZD55-IL-24, and ZD55-TRAIL, used separately except their combination, promoted cancer growth (i.e. increase of viability). The highest cancer-promoting effect was found in ZD55-IL-24 group (highest

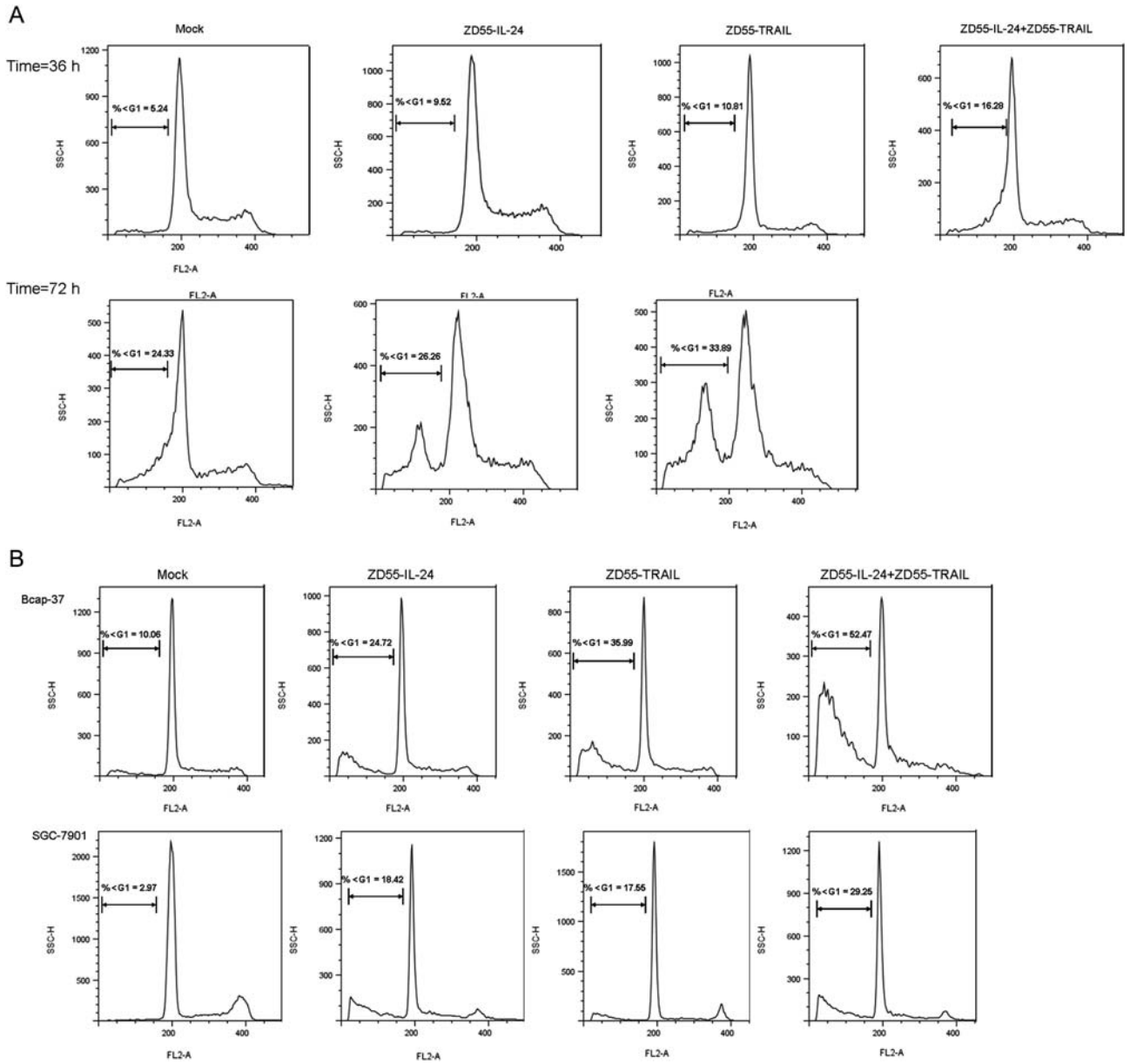


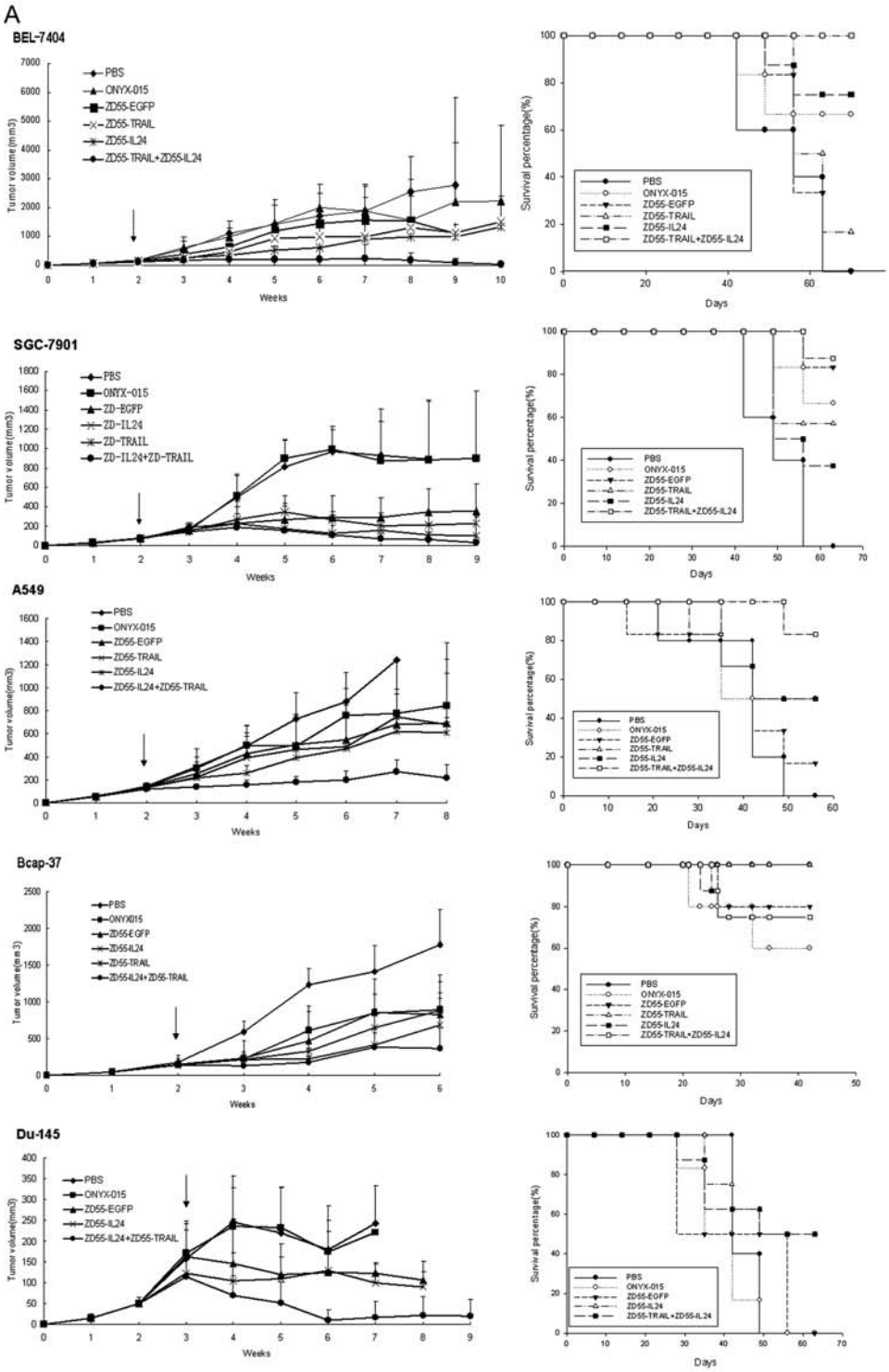
Figure 4 The apoptosis induced by ZD55-TRAIL and ZD55-IL-24 or their combination assayed by cytometry Different cancer cell lines were treated with ZD55-TRAIL and ZD55-IL-24 alone or in combination at five MOIs, after different times, then stained with PI and carried out flow cytometric analysis as shown in (A) is BEL 7404, 36 and 72 h, and in (B) Bcap37 and SGC-7901, all 72 h. The apoptotic cell percentage of combined treatment was higher than that of individual ZD55-TRAIL or ZD55-IL-24 treatment.

viability) and the least cancer-promoting effect was found in ZD55-TRAIL (least viability). Furthermore, ZD55-TRAIL could inhibit the ZD55-IL-24 cancer-promoting effect and cause the decrease of the cancer cell viability, which is time dependent, and strongly reduced the viability when used with a combination of ZD55-TRAIL and ZD55-IL-24, especially for Bcap37 at 96 h as shown in **Fig. 1(A)**. This is an important finding of this study. The gastric cancer cell line SGC-7901 and liver cancer cell line BEL7404 were also treated with different viruses at different MOI. It was found that all the combined use of ZD55-TRAIL and ZD55-IL-24 has stronger antitumor effect except ZD55-IL-24 with 1 MOI in BEL7404 as shown in **Fig. 1(B)**, which may be due to the cancer-promoting effect of ZD55-IL-24 itself as stated above.

In this study, we only used mock as control and studied the cancer cell line without normal cell line, as Fisher [26] has shown that IL-24 has no toxicity on 11 normal cell lines.

Induction of TRAIL expression by ZD55-IL-24

Different cancer cell lines were treated at 5 or 10 MOIs of ZD55-TRAIL and ZD55-IL-24, respectively, or five MOIs of ZD55-TRAIL and 5 MOIs of ZD55-IL-24 in



combination as indicated in **Fig. 2(B)**. The cell lysate was collected after 48-h infection to detect E1A, IL-24, and TRAIL. The bands of E1A and IL-24 were diverging because of their isomers, then their quantities were measured by densitometric analysis and showed in a column diagram. As shown in **Fig. 2(A)**, the E1A of adenovirus had not changed, which meant there is no effect on each other after different treatment. The expression of TRAIL could be induced by the treatment of five MOIs of ZD55-IL-24 in A549 cell line [**Fig. 2(B)**] and more were induced when 10 MOIs of ZD55-IL-24 was used as shown in **Fig. 2(B)**, indicating the up-regulation of TRAIL expression by ZD55-IL-24 treatment.

Antitumor effect of ZD55-TRAIL and ZD55-IL-24 or their combination by the induction of apoptosis mediated through caspase cascade

Previous studies showed that TRAIL induced cell apoptosis by activating caspase cascade [23] and IL-24 exerted its tumor-suppressive effect via different signaling pathways in different tumor types [25]. To determine the signaling pathway of apoptosis, western blot analysis for caspase cascade was conducted after treatment with ZD55-TRAIL and ZD55-IL-24 alone or in combination. Caspase-3, -8, -9, and poly (ADP-ribose) polymerase (PARP) were activated in cell line SGC-7901 [**Fig. 3(A)**]. Similar examinations were performed in cell lines BEL-7404, DU-145, and Bcap-37, the results were similar (data not shown), indicating that combined therapy induced both mitochondria (intrinsic) and non-mitochondrial (extrinsic) apoptosis pathway.

The apoptosis induced by ZD55-TRAIL and ZD55-IL-24 or their combination assayed by cytometry

Flow cytometric analysis (with PI staining) was used to measure the apoptosis induced by ZD55-TRAIL, ZD55-IL-24, or their combination in BEL-7404 for 36 or 72 h, using the untreated cell line as negative control [**Fig. 4(A)**]. The combined treatment with ZD55-TRAIL and ZD55-IL-24 induced apoptosis much more than ZD55-TRAIL or ZD55-IL-24 alone in human cancer cell lines Bcap-37 and SGC-7901 [**Fig. 4(B)**], similar results were also obtained in Du145 and A549 cell line (data not shown). As another control, the OV ONYX-015 only induced very little apoptosis (data not shown). These results suggested that the combined treatment of ZD55-TRAIL and ZD55-IL-24 induced additional apoptosis in several human cancer cell lines and then suppressed tumor growth dramatically.

Complete eradication of hepatoma xenograft by combined use of ZD55-TRAIL and ZD55-IL-24

Balb/c nude mice were used to establish different cancer models and treated by intratumor injection of ZD55-TRAIL and ZD55-IL-24 or their combination. ONYX-015 and ZD55-EGFP were also used as control in addition to PBS. The results are shown in **Fig. 5(A)**. Individual use of ZD55-TRAIL and ZD55-IL-24 could inhibit tumor growth compared with control. The antitumor effect of combined treatment was obviously the best one among all the experimental groups of all xenograft tumors, and the animal survival rate was also the highest [**Fig. 5(B)**]. Furthermore, in the combined therapy group of BEL-7404 liver cancer, all the xenograft tumors were completely eliminated and no nude mouse died in the combined treatment. The CTGVT-DG is a promising strategy for human cancer therapy including liver, gastric, colorectal, breast, lung, and prostate carcinomas.

Discussion

There are numerous protocols for the treatment of cancer patients, including chemotherapy, radiotherapy, gene therapy, virotherapy, and so on, but few of them can completely eliminate xenograft tumors. Our CTGVT, especially the CTGVT-DG may provide a new approach to eliminate inoculated tumors. ZD55, with the deletion of viral E1B-55KD, is similar to ONYX-015 which has reached Phase III clinical trial in the USA. It was reported in 1992 that E1B-55KD protein could bind P53 in normal cells to inhibit its transcriptional activation [27] and induce the degradation of P53 by cooperating with another viral protein E4-ORF6 [28]. But later, O'Shea *et al*. [29] reported that the late viral RNA export, rather than P53 inactivation, determined ONYX-015 (ZD55) tumor selectivity and heat shock protein (may be also HSP70) could mimic the function of E1B-55KD [30]. In 2010, O'Shea's lab found that a small viral protein E4-ORF3 deletion could make the ZD55 with more safety [31].

Figure 5 Complete eradication of hepatoma xenograft by the combined use of ZD55-TRAIL and ZD55-IL-24 Different cancer cell lines BEL-7404, SGC-7901, A549, Bcap-37, and DU-145 were inoculated at right flank of nude mice. When the tumor volume grew to 100–120 mm^3, mice were divided randomly into five groups, then different viruses were injected intratumorally every other day, four times with total amount of 2×10^9 PFU per mouse. (A) The tumor volume were measured weekly after CTGVT viruses injection and also recorded the animal survival percentage. The arrow indicated the time of treatment. For BEL-7404, SGC-7901, and Bcap-37 groups, $n = 8$; for A549 and Du145 groups, $n = 6$; for control groups (including PBS, ONYX-015 and ZD55-EGFP), $n = 6$. (B) Photo picture showed the complete elimination by the combined treatment of ZD55-TRAIL plus ZD55-IL-24 for BEL-7404 liver cancer.

In addition to the use of ZD55, many other OVs can be produced by replacing the native promoter of E1A or E1B with cancer-specific promoters such as hTERT, survivin, and α-fetoprotein promoters. So after inserting different antitumor genes into them, many CTGVT(s) can be constructed. All these CTGVTs have much better antitumor effects than that of either their respective gene therapy or oncolytic adenovirus therapy in our more than 60 peer-reviewed papers. Furthermore, CTGVT-DG strategy has much better antitumor effect than that of CTGVT with single gene, on account of compensative or synergistic effect of two genes. We have more than six papers reporting essentially complete elimination of xenograft tumor of different tissues [6–8, 32–34], among which the best one is the combined use of ZD55-TRAIL and ZD55-IL-24. There are two contributions of this paper: (i) the combination application of ZD55-TRAIL and ZD55-IL-24 showed good antitumor effect for several xenograft tumors, such as liver, gaster, lung, breast, and prostate carcinoma; (ii) the mutual up-regulating antitumor effect displaying that TRAIL could be induced by expression of ZD55-TRAIL [**Fig. 2(B)**] and ZD55-TRAIL in turn could increase the antitumor effect of ZD55-IL-24, especially in Bcap37 group at 96 h [**Fig. 1(A)**]. The synergistic antitumor effect of ZD55-TRAIL and ZD55-IL-24 is the basis for this potent antitumor effect. In addition, we have successfully combined ZD55-TRAIL and ZD55-IL-24 together by a four-amino acid linker IETD to form ZD55-TRAIL-IETD-ZD55-IL-24, which also showed a similar antitumor effect and may have a promising potential for clinical use.

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References

1 Liu XY. A new anticancer strategy–gene-virotherapy therapy of cancer. Chin J Cancer Biother 2001, 8: 1.
2 Zhang ZL, Zou WG, Luo CX, Li BH, Wang JH, Sun LY and Qian QJ, *et al*. An armed oncolytic adenovirus system, ZD55-gene, demonstrating potent antitumoral efficacy. Cell Res 2003, 13: 481–489.
3 Evans J. Recent deal highlights hopes for cancer-killing viruses. Nat Med 2011, 17: 268–269.
4 Breitbach CJ, Burke J, Jonker D, Stephenson J, Haas AR, Chow LQ and Nieva J, *et al*. Intravenous delivery of a multi-mechanistic cancer-targeted oncolytic poxvirus in humans. Nature 2011, 477: 99–102.
5 Zhao L, Gu J, Dong A, Zhang Y, Zhong L, He L and Wang Y, *et al*. Potent antitumor activity of oncolytic adenovirus expressing mda-7/IL-24 for colorectal cancer. Hum Gene Ther 2005, 16: 845–858.
6 Zhao L, Dong A, Gu J, Liu Z, Zhang Y, Zhang W and Wang Y, *et al*. The antitumor activity of TRAIL and IL-24 with replicating oncolytic adenovirus in colorectal cancer. Cancer Gene Ther 2006, 13: 1011–1022.
7 Zhang Y, Gu J, Zhao L, He L, Qian W, Wang J and Wang Y, *et al*. Complete elimination of colorectal tumor xenograft by combined manganese superoxide dismutase with tumor necrosis factor-related apoptosis-inducing ligand gene virotherapy. Cancer Res 2006, 66: 4291–4298.
8 Pei Z, Chu L, Zou W, Zhang Z, Qiu S, Qi R and Gu J, *et al*. An oncolytic adenoviral vector of Smac increases antitumor activity of TRAIL against HCC in human cells and in mice. Hepatology 2004, 39: 1371–1381.
9 Lebedeva IV, Sarkar D, Su ZZ, Kitada S, Dent P, Stein CA and Reed JC, *et al*. Bcl-2 and Bcl-x(L) differentially protect human prostate cancer cells from induction of apoptosis by melanoma differentiation associated gene-7, mda-7/IL-24. Oncogene 2003, 22: 8758–8773.
10 Lebedeva IV, Su ZZ, Chang Y, Kitada S, Reed JC and Fisher PB. The cancer growth suppressing gene mda-7 induces apoptosis selectively in human melanoma cells. Oncogene 2002, 21: 708–718.
11 Su ZZ, Madireddi MT, Lin JJ, Young CS, Kitada S, Reed JC and Goldstein NI, *et al*. The cancer growth suppressor gene mda-7 selectively induces apoptosis in human breast cancer cells and inhibits tumor growth in nude mice. Proc Natl Acad Sci USA 1998, 95: 14400–14405.
12 Saeki T, Mhashilkar A, Chada S, Branch C, Roth JA and Ramesh R. Tumor-suppressive effects by adenovirus-mediated mda-7 gene transfer in non-small cell lung cancer cell *in vitro*. Gene Ther 2000, 7: 2051–2057.
13 Patani N, Douglas-Jones A, Mansel R, Jiang W and Mokbel K. Tumour suppressor function of MDA-7/IL-24 in human breast cancer. Cancer Cell Int 2010, 10: 29.
14 Sauane M, Su ZZ, Gupta P, Lebedeva IV, Dent P, Sarkar D and Fisher PB. Autocrine regulation of mda-7/IL-24 mediates cancer-specific apoptosis. Proc Natl Acad Sci USA 2008, 105: 9763–9768.
15 Su Z, Lebedeva IV, Gopalkrishnan RV, Goldstein NI, Stein CA, Reed JC and Dent P, *et al*. A combinatorial approach for selectively inducing programmed cell death in human pancreatic cancer cells. Proc Natl Acad Sci USA 2001, 98: 10332–10337.
16 Su ZZ, Lebedeva IV, Sarkar D, Gopalkrishnan RV, Sauane M, Sigmon C and Yacoub A, *et al*. Melanoma differentiation associated gene-7, mda-7/IL-24, selectively induces growth suppression, apoptosis and radiosensitization in malignant gliomas in a p53-independent manner. Oncogene 2003, 22: 1164–1180.
17 Wiley SR, Schooley K, Smolak PJ, Din WS, Huang CP, Nicholl JK and Sutherland GR, *et al*. Identification and characterization of a new member of the TNF family that induces apoptosis. Immunity 1995, 3: 673–682.
18 Ashkenazi A, Pai RC, Fong S, Leung S, Lawrence DA, Marsters SA and Blackie C, *et al*. Safety and antitumor activity of recombinant soluble Apo2 ligand. J Clin Invest 1999, 104: 155–162.
19 Walczak H, Miller RE, Ariail K, Gliniak B, Griffith TS, Kubin M and Chin W, *et al*. Tumoricidal activity of tumor necrosis factor-related apoptosis-inducing ligand *in vivo*. Nat Med 1999, 5: 157–163.
20 Kagawa S, He C, Gu J, Koch P, Rha SJ, Roth JA and Curley SA, *et al*. Antitumor activity and bystander effects of the tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) gene. Cancer Res 2001, 61: 3330–3338.

21 Chinnaiyan AM, Prasad U, Shankar S, Hamstra DA, Shanaiah M, Chenevert TL and Ross BD, *et al*. Combined effect of tumor necrosis factor-related apoptosis-inducing ligand and ionizing radiation in breast cancer therapy. Proc Natl Acad Sci USA 2000, 97: 1754–1759.
22 Shi J, Zheng D, Liu Y, Sham MH, Tam P, Farzaneh F and Xu R. Overexpression of soluble TRAIL induces apoptosis in human lung adenocarcinoma and inhibits growth of tumor xenografts in nude mice. Cancer Res 2005, 65: 1687–1692.
23 Kischkel FC, Lawrence DA, Chuntharapai A, Schow P, Kim KJ and Ashkenazi A. Apo2L/TRAIL-dependent recruitment of endogenous FADD and caspase-8 to death receptors 4 and 5. Immunity 2000, 12: 611–620.
24 Lin T, Gu J, Zhang L, Huang X, Stephens LC, Curley SA and Fang B. Targeted expression of green fluorescent protein/tumor necrosis factor-related apoptosis-inducing ligand fusion protein from human telomerase reverse transcriptase promoter elicits antitumor activity without toxic effects on primary human hepatocytes. Cancer Res 2002, 62: 3620–3625.
25 National Research Council. Guide for the Care and Use of Laboratory Animals. Washington, DC: The National Academies Press, 1996.
26 Fisher PB. Is mda-7/IL-24 a 'magic bullet' for cancer? Cancer Res 2005, 65: 10128–10138.
27 Yew PR and Berk AJ. Inhibition of p53 transactivation required for transformation by adenovirus early 1B protein. Nature 1992, 357: 82–85.
28 Querido E, Blanchette P, Yan Q, Kamura T, Morrison M, Boivin D and Kaelin WG, *et al*. Degradation of p53 by adenovirus E4 or f6 and E1B55K proteins occurs via a novel mechanism involving a Cullin-containing complex. Genes Dev 2001, 15: 3104–3117.
29 O'Shea CC, Johnson L, Bagus B, Choi S, Nicholas C, Shen A and Boyle L, *et al*. Late viral RNA export, rather than p53 inactivation, determines ONYX-015 tumor selectivity. Cancer Cell 2004, 6: 611–623.
30 O'Shea CC, Soria C, Bagus B and McCormick F. Heat shock phenocopies E1B-55K late functions and selectively sensitizes refractory tumor cells to ONYX-015 oncolytic viral therapy. Cancer Cell 2005, 8: 61–74.
31 Soria C, Estermann FE, Espantman KC and O'Shea CC. Heterochromatin silencing of p53 target genes by a small viral protein. Nature 2010, 466: 1076–1081.
32 Zhang Z, Huang Y, Newman K, Gu J, Zhang X, Wu H and Zhao M, *et al*. Reexpression of human somatostatin receptor gene 2 gene mediated by oncolytic adenovirus increases antitumor activity of tumor necrosis factor-related apoptosis-inducing ligand against pancreatic cancer. Clin Cancer Res 2009, 15: 5154–5160.
33 Chu L, Gu J, He Z, Xiao T and Liu X. Adenoviral vector expressing CYLD augments antitumor activity of TRAIL by suppression of NF-kappaB survival signaling in hepatocellular carcinoma. Cancer Biol Ther 2006, 5: 615–622.
34 Liu XY, Qiu SB, Zou WG, Pei ZF, Gu JF, Luo CX and Ruan HM, *et al*. Effective gene-virotherapy for complete eradication of tumor mediated by the combination of hTRAIL (TNFSF10) and plasminogen k5. Mol Ther 2005, 11: 531–541.