Gene therapy for bladder cancer using E1B-55 kD-deleted adenovirus in combination with adenoviral vector encoding plasminogen kringles 1–5

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Mutations or loss of heterozygosity of p53 are detected in approximately 50% of bladder cancers. E1B-55 kD-deleted adenovirus has been shown to kill tumour cells with defective p53 function while sparing normal cells. Here, we examined the cytolytic effect and replication of E1B-55 kD-deleted adenovirus, designated Ad5WS1, on human bladder cancer cell lines with various p53 status. Ad5WS1 caused more severe cytolytic effect and replicated more efficiently in J82 and TCC-SUP bladder cancer cells carrying mutant p53 compared with TSGH-B301 and BFTC-905 bladder cancer cells retaining wild-type p53. Introduction of dominant negative p53 into BFTC-905 cells rendered them more susceptible to Ad5WS1-induced cytolysis. Furthermore, cells susceptible to lysis caused by Ad5WS1 were not attributable to their greater infectability by adenovirus. Finally, Ad5WS1 suppressed the growth of TCC-SUP bladder tumour xenografts, which could be augmented when combined with replication-defective adenoviral vector encoding kringles 1–5 of plasminogen (K1–5), an angiogenic inhibitor. Taken together, our results show that E1B-55 kD-deleted adenovirus replicates and hence lyases bladder cancer cells with mutant p53 much more efficient than those with wild-type p53. Thus, E1B-deleted adenovirus may have therapeutic potential, especially in combination with adenoviral vector expressing K1–5, for the treatment of bladder cancer.

British Journal of Cancer (2003) 88, 1492–1499. doi:10.1038/sj.bjc.6600908 www.bjcancer.com

Keywords: E1B-deleted; adenovirus; gene therapy; bladder cancer; p53; plasminogen; kringles 1–5

Transitional cell carcinoma (TCC) of the bladder is a common cause of death of genitourinary tumours. The generally poor prognosis of advanced bladder cancer indicates the need for new therapeutic modalities. Mutations in the p53 gene are the most common genetic defect in human bladder cancer, which occur in approximately 50% of bladder cancer and are associated with high-stage, high-grade TCC (Sidransky et al, 1991; Esrig et al, 1994). In accordance with the important role of p53 in bladder carcinogenesis, loss of heterozygosity of 17p, where p53 gene is located, is detected in more than 60% of patients with poorly differentiated tumours (Olumi et al, 1990). There is a significant correlation between the mutations in the p53 gene and increased microvessel counts in patients with invasive bladder cancer (Bochner et al, 1997). Furthermore, p53 exerts its tumour suppressor effects, in part, through the control of bladder tumour angiogenesis (Grossfeld et al, 1997). Therefore, angiogenesis may be one of the therapeutic targets for treating bladder cancer.

The E1B-55 kD protein of adenovirus is able to bind and inactivate p53, and thus allows adenovirus to replicate in cells without the restrain of p53 growth control. Bischoff et al (1996) first reported that E1B-55 kD-deleted adenovirus selectively replicated in and lysed cancer cells lacking functional p53 while sparing normal cells. However, subsequent investigations have suggested that replication of this mutant adenovirus may not be entirely dependent upon p53 status (Rothmann et al, 1998; Harada and Berk, 1999). Despite controversy concerning the mechanisms underlying the replication of E1B-55 kD-deleted adenovirus in tumour cells, it is currently employed for cancer therapy, and clinical trials have been promising, especially combined with chemotherapy or radiotherapy (Khuri et al, 2000; Habib et al, 2001). Notably, no virus was detected in the normal tissue examined in clinical trials (Khuri et al, 2000).

Targeting angiogenesis to kill tumour cells is one of the promising therapeutic approaches for cancer. Angiostatin comprising the first four kringles of plasminogen is an endogenous inhibitor of angiogenesis that inhibits the growth of primary and metastatic tumours (O’Reilly et al, 1994). A plasminogen fragment containing kringles 1–5 (K1–5) has been shown to exhibit higher antiangiogenic activity and hence higher antitumour effect compared with angiostatin (Cao et al, 1999). Targeting tumour vasculature with antiangiogenic gene therapy has become a promising new approach for cancer therapy.
Based on the fact that most bladder cancers have mutation in the p53 gene, in this study we examined the cytolytic effect of E1B-55 kD-deleted adenovirus on bladder cancer cells with various p53 status. We demonstrate that this mutant adenovirus exerted a p53-dependent cytolytic effect on bladder cancer cells and its antitumour effect could be augmented when combined with E1-deleted adenoviral vector expressing K1–5. Therefore, our results suggest that E1B-55 kD-deleted adenovirus may have therapeutic potential, especially in combination with replication-defective adenoviral vector expressing K1–5, for the treatment of patients with bladder cancer.

**MATERIALS AND METHODS**

**Cell lines and mice**

TSGH-8301 (Yeh et al., 1988) and BFTC-905 (Tseng et al., 1996) cells carry wild-type p53, while J82 and TCC-SUP cells harbour mutant p53 (Rieger et al., 1995; Markl and Jones, 1998). BFTC-173-12 cells were derived from BFTC-905 cells by transfection with V173L, an expression plasmid of a dominant-negative mutant p53 with a substitution of leucine for valine at codon 173 (Chen et al., 1993), followed by selection of stable transfectants with G418 (Chang and Lai, 2001). All the bladder cancer and 293 cells were cultured in Dulbecco’s modified Eagle’s medium containing 10% fetal bovine serum, 2 mM glutamine and 50 μg ml⁻¹ gentamicin. Female BALB/cBy SmnPkd SCID mice at the age of 6–10 weeks used for TCC-SUP tumour cell implantation were obtained from the Laboratory Animal Center of the National Cheng Kung University. The animals were maintained in specific pathogen-free animal care facility under isothermal conditions with regular photoperiods. The experimental protocol adhered to the rules of the Animal Protection Act of Taiwan and was approved by the Laboratory Animal Care and Use Committee of the National Cheng Kung University, in full accordance with the UK Guidelines for the Welfare of Animals in Experimental Neoplasia (Workman et al., 1998).

**Construction of recombinant adenoviruses**

The E1B-55 kD-deleted adenovirus, designated Ad5WS1, was constructed as described previously (Hsrie et al., 2003). The E1-deleted adenoviral vector encoding K1–5, designated Ad/K1–5, was constructed as follows. The coding region of murine K1–5 was generated from pRR1/plasminogen (ATCC, Manassas, VA, USA) by PCR with sense primer 5'-CCC AAG CTT GGA TCC CAT GAA -3' and antisense primer 5'-GCT CTA GAA -3' sites, and cloned into the HindIII site of a plasmid expressing cells by the total number of cells from three separate microscopic fields at a magnification of ×200.

**Determination of infectability of human bladder cancer cell lines by adenovirus**

To determine the infectability by adenovirus, bladder cancer cells cultured in 24-well plates were infected with various doses of Ad/LacZ in triplicate and incubated for 48 h, after which the cells were stained for β-gal activity (Hashimoto et al., 1997). Infectability was expressed as a percentage by dividing the number of β-gal-expressing cells by the total number of cells from three separate microscopic fields at a magnification of ×200.

**Chorioallantoic membrane (CAM) assay**

The angiogenesis assay was carried out as previously described (Shing et al., 1985). Ad/K1–5 or Ad/LacZ at a dose of 2 × 10⁷ plaque-forming units (PFU), or PBS was implanted onto the CAMs of a 6-day-old chick embryo through a window made in the egg shell. The CAMs were photographed and analysed for angiogenesis after incubation for 24 h at 37°C.

**Immunohistochemical and vascular staining**

At 48 h after infection with Ad/K1–5 at an MOI of 2, TCC-SUP cells were fixed in 3.7% formaldehyde, treated with cold acetone, and quenched in 3.7% H₂O₂ for detection of K1–5 expression by immunohistochemical staining using rabbit antibody against human plasminogen kringle 1–3 (K1–3). To evaluate K1–5 expression in tumours, TCC-SUP cells (10⁷ cells) were inoculated subcutaneously (s.c.) into the flank of SCID mice at day 0. At day 10, mice were injected intratumorally with Ad/K1–5 at a dose of 10⁸ PFU for 5 consecutive days. At day 17, tumours were excised and snap frozen. Cryostate sections (4 μm) were prepared and incubated with rabbit antibody against K1–3 followed by detection with the DAKO LSAB 2 System (Dako, Carpinteria, CA, USA) according to the manufacturer’s instructions. Next, the sections were incubated with biotinylated secondary antibody, horseradish peroxidase-labelled streptavidin, and aminoethyl carbazole (AEC) as substrate chromogen, the slides were counterstained with haematoxylin. For vascular staining, the slides were incubated with polyclonal anti-factor VIII (von Willebrand’s factor, DAKO), peroxidase-labelled goat anti-rabbit IgG (H & L chain specific, Calbiochem, Darmstadt, Germany), followed by application of AEC, and counterstaining with haematoxylin. Stained blood vessels were counted in three blindly chosen random fields at ×40 magnification, and the mean and s.d. of the counts were calculated.

**Animal studies**

TCC-SUP cells (10⁴) were inoculated s.c. into the flank of SCID mice at day 0. At day 8, visible and palpable nodules developed at 25.08 ± 47.71 mm³, with mean tumour volume of 31.89 ± 6.45 mm³. Groups of seven mice were treated intratumorally with Ad5WS1 (10⁸ PFU in 100 μl of PBS) or Ad/K1–5 (2.5 × 10⁷ PFU in 100 μl of PBS) alone for 5 consecutive days, or with Ad5WS1 (10⁸ PFU in 100 μl of PBS) at days 8, 10 and 12 plus Ad5WS1 (10⁶ PFU in 50 μl of PBS) admixed with Ad/K1–5 (2.5 × 10⁸ PFU in 50 μl of PBS) at days 9 and 11. As the injection volume of adenovirus correlates with intratumoral virus distribution, and hence influences therapeutic efficacy (Heise et al., 1999), Ad5WS1 infection at an MOI of 2 by trypan blue exclusion. Viral replication was assayed as previously described (Hsrie et al., 2003). Briefly, confluent cells in six-well plates were infected with Ad5WS1 at an MOI of 2. Viruses harvested from both the supernatant and cell lysate at 26 h postinfection were pooled and titered on 293 cells by 50% tissue culture infective dose (TCID₅₀) method.

**Assays of cytopathic effect (CPE), cell viability and viral replication**

Cells cultured in six-well plates were infected with Ad5WS1 at 0.001, 0.01, 0.1 and 1 multiplicities of infection (MOIs) for 2 h. Cells were observed daily for CPE. Once the CPE was evident, which took 5–9 days, cells were stained with 10% formalin/0.05% crystal violet solution. The viable cell numbers were also determined in cells seeded in six-well plates after 3 days of
in this study the volume for each intratumoral injection was fixed to 100 µl. All mice were monitored for tumour growth after tumour inoculation. Palpable tumours were measured once a week in two perpendicular axes with a tissue caliper and the tumour volume was calculated as (length of tumour) × (width of tumour)^2 × 0.45. The mice were killed by intraperitoneal injection of pentobarbital at day 58, when tumours reached approximately 1.7 cm in mean diameter in PBS-treated group.

Statistical analysis

Tumour volumes and vessel density were compared using an unpaired, two-tailed Student’s t-test. Any P-values less than or equal to 0.05 were regarded as statistically significant.

RESULTS

CPE in bladder cancer cells infected with Ad5WS1

The cytolytic effect of Ad5WS1 on four bladder cancer cell lines with different p53 status was examined. At an MOI of 2, Ad5WS1 caused CPE in J82 and TCC-SUP cells carrying mutant p53, but not in TSGH-8301 and BFTC-905 cells retaining wild-type p53 at 5 days postinfection (Figure 1). At 7 days after infection, Ad5WS1 completely lysed 293 cells at MOIs ranging from 0.01 to 10 (Figure 2A). It lysed TCC-SUP and J82 cells in a dose-dependent manner, with complete cytolysis at an MOI of 10 at 5–7 days postinfection (Figure 2B and C). Nevertheless, even when monitored for 9 days, no significant CPE was observed in TSGH-8301 and BFTC-905 cells infected with Ad5WS1. Of note, viral yield produced in J82 cells was found to be 10.34-fold higher over that in 293 cells. However, viral replication was only 0.87% and 1.52% in TSGH-8301 and BFTC-905 cells, respectively, as compared with that in 293 cells. Collectively, Ad5WS1 replicated more efficiently in bladder cancer cells with mutant p53 than in those with wild-type p53.

Replication of Ad5WS1 in bladder cancer cells

The ability of Ad5WS1 to replicate in four bladder cancer cell lines was assessed at 26 h postinfection. As shown in Figure 3, Ad5WS1 replicated and produced high titers on both J82 and TCC-SUP cells. Viral yield did not differ between TCC-SUP and 293 cells infected with Ad5WS1. Of note, viral yield produced in J82 cells was found to be 10-fold higher over that in 293 cells. However, viral replication was only 0.87% and 1.52% in TSGH-8301 and BFTC-905 cells, respectively, as compared with that in 293 cells. Collectively, Ad5WS1 replicated more efficiently in bladder cancer cells with mutant p53 than in those with wild-type p53.

CPE in mutant p53 transfectant infected with Ad5WS1

Further evidence to support the notion that mutation of p53 in cells renders them more sensitive to Ad5WS1-induced cytolysis is presented in Figure 4. BFTC-173-12 cells, which harboured dominant-negative p53, exhibited severe CPE following Ad5WS1 infection (Figure 4A), whereas no significant CPE was noticed in parental BFTC-905 cells (Figure 1). In BFTC-173-12 cells infected with Ad5WS1, the number of viable cells decreased to 50% at 3 days postinfection compared with mock-infected cells, whereas the viability of Ad5WS1-infected BFTC-905 cells was not significantly different from mock-infected cells (Figure 4B). In terms of virus replication, viral yields in BFTC-173-12 and BFTC-905 cells were $1.58 \times 10^6$ and $1.58 \times 10^7$ TCID$_{50}$ ml$^{-1}$ at 26 h postinfection, respectively. These results demonstrate that the mutant p53 transfectant was more permissive for Ad5WS1 replication than parental cells with wild-type p53, thus resulting in more cytolysis.

Infectability of bladder cancer cells by adenovirus

The observed differences in the susceptibility of various human bladder cancer cells to Ad5WS1-induced cytolysis could be attributable to various levels of cell infectability by adenovirus among different cell lines. To address this question, we examined the infectability of the cells by Ad/LacZ, a recombinant adenoviral vector carrying the β-gal reporter gene. As shown in Figure 5, β-gal expression was detectable, albeit at different levels, in all of the cells examined after exposure to Ad/LacZ. Furthermore, the infectability of the cells increased when higher doses of Ad/LacZ were applied. The most notable difference was found in TSGH-8301 cells that exhibited three times more infectable with adenovirus compared with J82 cells, which were the least infectable cells. The infectability between BFTC-173-12 and BFTC-905 did not differ, suggesting that introduction of dominant-negative p53 in the BFTC-905 cells, to obtain the stable BFTC-173-12 transfectant, did not change the infectability of the cells compared with the parental cells.

Functional characterisation of K1–5 expressed by Ad/K1–5

Expression of K1–5 was detectable in more than 80% of TCC-SUP cells transduced with Ad/K1–5 (Figure 6A) compared with untransduced cells (Figure 6B). Expression of K1–5 in tumours was further examined in TCC-SUP tumour-bearing SCID mice treated with Ad/K1–5. By immunohistochemical staining, K1–5...
protein was detectable in the tumour xenografts from Ad/K1–5-treated (Figure 6C), but not PBS-treated mice (Figure 6D). Furthermore, tumour xenografts were analysed for microvessel morphology and vascularisation by staining for factor VIII. Tumour sections from Ad/K1–5-treated mice appeared much less vascularised (Figure 6E and G) than their PBS-treated counterparts (Figure 6F and H). The vessel density underneath tumour capsules was markedly reduced in Ad/K1–5-treated (12.7 ± 2.65 vessels per field) compared with PBS-treated (32.67 ± 2.52 vessels per field) tumours (P < 0.001). Furthermore, K1–5 encoded by Ad/K1–5 inhibited angiogenesis in the CAM assay. Ad/K1–5 inhibited the growth of embryonic new blood vessels (Figure 7A) compared with Ad/LacZ-treated (Figure 7B) or PBS-treated (Figure 7C) CAMs. Ad/K1–5 inhibited angiogenesis in four of six CAMs, while only one of six CAMs in Ad/LacZ-treated group revealed suppression of neovascularization, and none was found in PBS-treated CAMs. Taken together, these results indicate that the expressed K1–5 mediated by Ad/K1–5 gene transfer was detectable and displayed antiangiogenic activity in vitro and in vivo.
Antitumour effects of Ad5WS1 and Ad/K1–5
The antitumour effects of Ad5WS1 alone or combined with Ad/K1–5 were examined in pre-established human TCC-SUP bladder tumour xenografts. As shown in Figure 8, TCC-SUP tumours from either Ad5WS1-treated (P = 0.00027) or Ad/K1–5-treated (P = 0.00024) mice were significantly smaller than those from PBS-treated mice. Furthermore, at day 58 combination treatment of Ad5WS1 and Ad/K1–5 inhibited tumour growth compared with Ad5WS1-treated (P = 0.05) or Ad/K1–5-treated (P = 0.0018) mice. Of note, complete tumour regression was observed in one of seven established tumours in the combined treatment group, but not in the remaining three treatment groups (data not shown).

DISCUSSION
In the work described here, Ad5WS1 caused more severe CPE and produced higher viral yield in cells carrying mutant p53 compared with those carrying wild-type p53. At an MOI equal to or less than 2, the CPE caused by Ad5WS1 in human bladder cancer cell lines appeared to be p53-dependent. However, at very high viral doses and an extended period of infection, Ad5WS1 also caused some CPE, albeit only to a very small extent, in BFTC-905 and TSGH-8301 cells with wild-type p53. Studies along this line demonstrated that E1B-deleted adenovirus can infect tumour cells with functional p53 at higher viral doses, while kill those carrying nonfunctional p53 at much lower doses in hepatocellular carcinoma and lung cancer cell lines (Vollmer et al, 1999; You et al, 2000). It has been demonstrated that when sufficient E1B-55 KDa-deleted viral particles are delivered and sufficient target cells are in the S phase of the cell cycle, the virus may replicate regardless of the p53 status of target cells (Goodrum and Ornelles, 1997). In the viral replication assay shown here, compared with cancer cells harbouring wild-type p53, the observed CPE caused by Ad5WS1 in bladder cancer cells carrying mutant p53 corresponded to as much as 1000-fold difference in virus yield. Our results also indicate that infectious E1B-deleted adenoviruses may still be produced to a small extent in cells harbouring wild-type p53, despite the absence of an obvious CPE, which were in accordance with previous findings (Bischoff et al, 1996; Ganly et al, 2001). Since most related reports do not include quantitative evidence of viral replication and the production of new virus in the cancer cell lines studied, the relation between p53 status and viral replication or CPE still awaits clarification.

The infectability of cell lines by adenovirus has been reported to be dependent on the presence of coxsackie and adenovirus receptor (CAR) and αvβ integrins, which are two cell membrane components involved in viral attachment and internalisation (Wickham et al, 1993; Bergelson et al, 1997). It has been shown that a wide spectrum of CAR levels exists among human bladder cancer cells, which may correlate with their sensitivity to adenovirus infection (Li et al, 1999). Our results argue against the possibility that the infectability of the human bladder cancer cell lines by adenovirus contribute to their susceptibility to cytolysis induced by Ad5WS1. In this study, Ad5WS1 replicated efficiently and caused cytolysis in TCC-SUP but not in BFTC-905.
E1B-deleted adenovirus in bladder cancer
J-L Hsieh et al

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Figure 7  Chorioallantoic membrane (CAM) assay of Ad/K1–5. Chorioallantoic membranes of 6-day chick embryos were treated with (A) Ad/K1–5 (2 × 10⁷ PFU), (B) Ad/LacZ (2 × 10⁷ PFU) or (C) PBS. The CAMs were photographed after incubation for 24 h at 37°C.

Figure 8  Effects of Ad5WS1 and Ad/K1–5, singly or in combination, on human TCC-SUP bladder tumour xenograft growth. TCC-SUP cells (10⁷) were inoculated s.c. into the flank of SCID mice at day 0. At day 8 when visible and palpable nodules developed at all injection sites, groups of seven mice were treated intratumorally with Ad5WS1 (10⁸ PFU) or Ad/K1–5 (2.5 × 10⁸ PFU) alone for 5 consecutive days, or with Ad5WS1 (10⁷ PFU) for 5 consecutive days plus Ad/K1–5 (2.5 × 10⁸ PFU) at days 9 and 11. Data are presented as mean ± s.e.

of infectability, because BFTC-173-12 cells were as infectable by Ad/LacZ as were the parental BFTC-905 cells. To this end, we have recently shown that Ad5WS1-induced cytolysis is dependent on the transcription activity of p53 in hepatocellular carcinoma cells (Hsieh et al, 2003). Furthermore, introduction of the hepatitis B virus X gene into liver cells sensitisises them to cytolysis induced by Ad5WS1 through the disruption of p53 transcription activity.

Although antitumour activity of E1B-55 kD-deleted adenovirus has been demonstrated in a wide range of different human tumours in vitro and in vivo, the mechanism underlying its oncolytic activity remains controversial. Recently, E1B-55 kD-deleted adenovirus has been shown to exert cytotoxic effect in seven established human bladder cancer cell lines (van der Poel et al, 2002) in vitro. However, this study did not further investigate the underlying mechanism. Ries et al (2000) have shown that loss of p14ARF, a negative regulator for the MDM2 expression, facilitates the replication of E1B-55 kD-deleted adenovirus in tumour cells retaining wild-type p53. This finding supports the therapeutic use of this mutant adenovirus not only in tumours with mutant p53 but also in tumours with lesions within the p53 pathway. In bladder cancer, alterations affecting the p53 pathway, including p53, MDM2, p21WAF1, and p14ARF, are frequent events, which may be important diagnostic and prognostic markers in patients with bladder cancer (Lianes et al, 1994; Markl and Jones, 1998). As MDM2 ubiquitinates p53 and targets it for proteosomal degradation, it inactivates the function of p53. In a number of cancers, MDM2 is amplified or overexpressed, leading to inadequate levels of p53. Thus, p53 may be indirectly inactivated by mechanisms other than p53 mutations in some bladder cancers. It is plausible that tumour cells in which high levels of MDM2 suppress p53 may allow E1B-deleted adenovirus replication. BFTC-905 cells have been shown to overexpress MDM2 mRNA (Cheng et al, 1995), which may explain in part why Ad5WS1 at high doses caused CPE, albeit to a very small extent, in bladder cancer cells harbouring wild-type p53. Further analysis of the p53 pathway in bladder cancer cells may answer part of this question. The molecular basis for the growth differences of E1B-deleted adenovirus within different tumour and normal cell types remains to be determined.

In a number of clinical trials, E1B-55 kD-deleted adenovirus is well-tolerated by numerous routes of administration. However, as a single agent, its therapeutic efficacy for refractory solid tumours has been limited. Combination therapy seems to have greater promise in improving its antitumour activity. In the present study, we employed Ad5WS1 in combination with Ad/K1–5, an E1-deleted, replication-defective adenoviral vector expressing angiogenic inhibitors, for the treatment of TCC-SUP bladder tumour xenografts. Because antiangiogenesis therapy is likely to be most effective in a low tumour burden state (Bergers et al, 1999), we started the treatment in mice bearing tumours ranging approximately from 25 to 48 mm². Mice with low tumour burdens have been used in previous reports for evaluation of adenovirus-mediated antiangiogenic gene therapy (Im et al, 1999; Sauter et al, 2003).
2000). We found that combined therapy of AdSWS1 and Ad/K1−5 was superior to either treatment alone in inhibiting tumour growth. E1B-55 K-deleted adenovirus has been shown to act as a helper virus and augment the expression of the accompanying cytokine genes delivered by replication-defective adenoviral vector (Motoi et al, 2000). Although antiangiogenic therapy targets genetically stable endothelial cells in the tumour vasculature, tumour cells with mutated p53 are less susceptible to apoptosis under hypoxic conditions, resulting in reducing their reliance on vascular supply, and hence their responsiveness to antiangiogenic therapy (Yu et al, 2002). As tumour cell populations may be heterogeneous in terms of genetic mutations in clinical settings, combination therapy targeting to different types of tumour cells would be expected to enhance therapeutic efficacy. To this end, the strategy of combination therapy of oncolytic E1B-deleted adenovirus with adenovector encoding angiogenic inhibitors is more desirable in clinical settings, as cancer cells with functional p53 would be more susceptible to AdSWS1-induced cytolsis than those with wild-type p53, while tumours retaining functional p53 more responsive to antiangiogenic therapy because of more vascular dependence. Several phase I/II clinical trials of oncolytic adenoviruses for head and neck, pancreatic, ovarian and gastrointestinal carcinomas have been carried out. However, oncolytic adenoviral therapy has not been applied to the patients of more vascular dependence. Several phase I/II clinical trials of oncolytic adenoviruses for head and neck, pancreatic, ovarian and gastrointestinal carcinomas have been carried out. However, oncolytic adenoviral therapy has not been applied to the patients with bladder cancer. Owing to the fact that intact urothelium can function as an effective barrier, bladder is suitable for intravesical oncolytic adenoviral therapy has not been applied to the patients with bladder cancer. (Kuball et al, 2002).

In conclusion, our results demonstrate that E1B-55 K-deleted adenovirus caused more severe cytolytic effect and replicated more efficiently in bladder cancer cells with mutant p53 than in those with wild-type p53. In the bladder xenograft animal model, it also exerted antitumour effects, which could be augmented by combining with replication-defective adenoviral vector expressing K1−5. Therefore, our results suggest that E1B-55 K-deleted adenovirus may have therapeutic potential, especially in combination with adenoviral vector expressing K1−5, for the treatment of patients with bladder cancer.

ACKNOWLEDGEMENTS

We are indebted to Dr MT Lin (Department of Biochemistry, NCKU) for providing polyclonal antibody against human K1−3. This work was supported by grants from the National Science Council (NSC 89-2318-B-006-014-M51 and NSC 90-2318-B-006-006-M51 to ALS, and NSC 89-2318-B-006-013-M51 and NSC 90-2318-B-006-003-M51 to CLW), Taiwan.

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