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A conditionally replicating adenovirus carrying interleukin-24 sensitizes melanoma cells to radiotherapy via apoptosis

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ABSTRACT

Combinatorial therapy is the current trend of the development of novel cancer treatments due to the high heterogeneous nature of solid tumors. In this study, we investigated the effects of the combined use of a conditionally replicating adenovirus carrying IL-24 (ZD55-IL-24) and radiotherapy on the proliferation and apoptosis of melanoma A375 cells in vitro and in vivo. Compared with either agent used alone, ZD55-IL-24 combined with radiotherapy significantly inhibited cell proliferation, accompanied with increased apoptosis. Radiotherapy did not affect the expression of IL-24 and E1A of ZD55-IL-24-treated cells, but increased the expression of Bax, promoted the activation of caspase-3, while decreasing Bcl-2 levels. Thus, this synergistic effect of ZD55-IL-24 in combination with radiotherapy provides a novel strategy for the development of melanoma therapies, and is a promising approach for further clinical development.

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1. Introduction

Malignant melanoma is one of the most lethal and aggressive human malignancies (Jemal et al., 2007). Malignant melanoma is commonly treated with a combination of therapies including surgical removal, chemotherapy, and radiotherapy. The long-term survival rate of melanoma is disappointing, in great part due to the resistance of melanoma cells to radio- or chemotherapy-induced apoptosis (Eberle et al., 2007; Jin et al., 2005). Given the limitations of the current therapies for metastatic melanoma, there is an urgent need for developing novel treatments to overcome acquired drug resistance.

Previous data suggest that interleukin-24 (IL-24) is a promising candidate for cancer gene therapy (Emdad et al., 2009). IL-24 suppresses growth and induces apoptosis in a wide range of human cancers without apparent cytotoxicity to normal cells (Emdad et al., 2009; Fisher, 2005; Fisher et al., 2007). Recent studies show that delivery of IL-24 by a replication incompetent adenovirus, Ad-IL-24, radiosensitizes various cancer cells, such as non-small cell lung carcinoma, renal carcinoma and malignant glioma (Eager et al., 2008). However, a major limitation of Ad-IL-24 is the inadequacy of replication-defective Ad vectors to efficiently infect tumor cells (Chu et al., 2004; Qian et al., 2008).

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1: PBS; 2: Radiation; 3: ZD55-IL-24; 4: ZD55-IL-24+ Radiation

Figure 1 – ZD55-IL-24 induced efficient delivery of IL-24 in melanoma cells. Melanoma cells were treated with ZD55-IL-24 plus radiation, ZD55-IL-24, radiation and PBS, respectively, for 48 h. IL-24 and E1A protein levels were analyzed by western blots using anti-IL-24 and E1A antibodies. β -actin was used as loading controls.

Conditionally replicating adenoviruses (CRAds) present a novel class of therapeutic agents in cancer treatment (Cody and Douglas, 2009). A prior study showed that the E1B 55-kDa gene-defective CRAd ZD55 not only efficiently infects, replicates in and lyses tumor cells, but also amplifies the IL-24 gene, accompanied with enhanced expression and function of IL-24 in the tumor microenvironment without affecting adjacent normal cells (Zhao et al., 2005). These advantages over replication-defective adenoviruses stimulate the wide interest in the development of CRAd-mediated gene therapies (Rein et al., 2006). In the past few years, a therapeutic regimen combining radiation with CRAds has shown great promise in cancer treatment (Freytag et al., 2007; Idema et al., 2007; Kim et al., 2009). An E1B 55 kDa-deleted CRAd, ONYX-015, has been shown to potentiate radiation therapy, leading to several pre-clinical studies examining the value of combining these

two modalities (Geoerger et al., 2003; Rogulski et al., 2000). Intra-arterial injections of ONYX-015, in combination with total body irradiation (5 Gy) result in enhanced anti-tumor efficacy in p53 functional human malignant glioma xenograft models (Geoerger et al., 2003). Furthermore, several specific CRAds, Ad5- Δ 24RGD, CV706, and Ad5-CD/TKrep in combination with radiotherapy were shown to have greater anti-tumor efficacy than either individual modality (Chen et al., 2001; Freytag et al., 2003; Lamfers et al., 2002).

These recent studies triggered us to hypothesize that a combination of ZD55-IL-24-mediated gene therapy with radiation therapy would produce an enhanced anti-tumor effect against melanoma in comparison with either agents used alone. Our data suggest that ZD55-IL-24 in combination with radiotherapy can be used as a potential therapeutic strategy for the treatment of this malignancy.

2. Materials and methods

2.1. Cell lines and vectors

The human melanoma A375 cell line was purchased from Shanghai Cell Collection (Shanghai, China). Cells were cultured in DMEM (GIBCO BRL, Grand Island, NY, USA) supplemented with 10% of heat-inactivated fetal bovine serum (FBS, GIBCO BRL), 4 mM glutamine, 50 U/mL penicillin and 50 μ g/mL streptomycin at 37 °C in a humidified atmosphere with 5% CO₂. The cells were screened routinely to verify lack of mycoplasma contamination for using in the long phase of growth. The conditionally replicating adenovirus ZD55

1: 2Gy Radition; 2: 4Gy Radition; 3: 8Gy Radition; 4: 0.1MOI ZD55-IL-24; 5: 1MOI ZD55-IL-24; 6: 10MOI ZD55-IL-24; 7: 0.1MOI ZD55-IL-24+2Gy Radition; 8: 1MOI ZD55-IL-24+4Gy Radition; 9: 10MOI ZD55-IL-24+8Gy Radition

Figure 2 – Combination of ZD55-IL-24 and radiotherapy inhibited the growth of melanoma cells. Melanoma cells were treated with ZD55-IL-24 plus radiation, ZD55-IL-24 and radiation at indicated dosages. The cells were subjected to MTT assay on the 4th day after treatment. Results are expressed as mean $\pm$ SD (error bars) by the percentage of untreated control cells. The asterisk “*” indicates $p < 0.05$ versus ZD55-IL-24, or radiation treated groups ($n = 6$).

Figure 3 – Combining ZD55-IL-24 with radiotherapy efficiently tilts the balance of Bcl-2 family proteins toward the pro-apoptotic pathway. (A) Melanoma cells were treated with ZD55-IL-24 plus radiation, ZD55-IL-24 and radiation, and PBS treatment was used as a negative control. After treatment for 72 h, cell extracts were subjected to Western blot analysis for activation of Caspase-3. The anti-apoptotic protein Bcl-2, and the pro-apoptotic protein Bax expression levels were also examined. β -actin was used as loading controls ($n = 6$). (B) Quantitation of changes in band densities by Image-J analysis software.

carrying IL-24 (ZD55-IL-24) used in this study was as previously described (Zhao et al., 2005, 2006). The expansion, purification, titration and quality analyses of those viruses were performed at the vector core facility of our institute.

2.2. Radiation

An X-irradiator (Model X.S.S.205 FZ, Jiancheng Co. LTD., China) was used in this study. The dose rate we used was 0.287 Gy/min with 200 kV/10 mA and filters of 0.5-mm-thick Cu/0.5-mm-thick Al, and the distance between the X-ray source and target was 56 cm. Cells were irradiated with 0.5–6 Gy of ionizing radiation using a ^{137}Cs g-irradiation source 48 h after viral treatment. For the tumor-bearing mice, X-rays were delivered in a focal manner to the tumor tissue with shielding of the other parts of the body after viral treatment.

2.3. Cell viability assay

A375 cells were plated at a same density of 10^5 cells/6 cm dish and treated with radiation alone with different dosages (2 Gy, 4 Gy, 8 Gy), and ZD55-IL-24 alone (0.1, 1, 10 MOI) and ZD55-IL-24 plus radiation (0.1MOI + 2 Gy, 1MOI + 4 Gy, 10MOI + 8 Gy) for the indicated time. After treatment for 4 days with the indicated dosage, cell survival rate was evaluated by standard MTT assay (Sigma, St. Louis, MO) according to the

manufacturer's protocol. Four replicate wells were tested per assay and each experiment was repeated three times.

2.4. Western blot analysis

Cells were harvested from the plates and aliquots of cell extracts were separated on a 12% SDS–polyacrylamide gel. The proteins were then transferred to nitrocellulose membrane and incubated overnight at 4 °C with the following rabbit polyclonal antibodies: anti-IL-24 (Gen Hunter C-corporation, Nashville, TN, USA), anti-Caspase-3, anti-Bcl-2, anti-Bax (Cell signaling, Beverly, MA, USA), anti- β -actin and anti-E1A antibodies (Santa Cruz Biotechnology, Santa Cruz, CA), respectively. Membranes were then washed and incubated with alkaline phosphatase conjugated secondary antibodies in TBST for 2 h and developed using NBT/BCIP color substrate (Promega, Madison, USA). The density of the bands on the membrane was scanned and analyzed with an Image-J analyzer (LabWorks Software, UVP Upland, CA, USA).

2.5. Immunocytochemical staining

Cells were fixed with 4% paraformaldehyde onto glass coverslips. After washing with phosphate-buffered saline (PBS), the cells were incubated with anti-Bax or anti-Bcl-2 antibody for 24 h, and then incubated with horseradish peroxidase-conjugated secondary antibody for 1 h followed by colorimetric

Figure 4 – The expression of Bcl-2 and Bax in melanoma cells was assessed by immunohistochemistry analysis. (A) Representative pictures for different treatment groups. (B) ZD55-IL-24 plus radiotherapy significantly reduced the number of Bcl-2-positive cells and induced the number of Bax-positive cells (Original magnification $\times 400$) ($n = 6$).

detection using diaminobenzidine (DAB). For evaluation of Bax and Bcl-2-positive fractions, at least 200 cells were counted from six different regions and the mean number was determined.

2.6. Xenograft tumor model in nude mice animals

Male BALB/c nude mice at 4–5 weeks old were obtained from the Shanghai Experimental Animal Center of the Chinese Academy of Sciences (Shanghai, China) and quarantined for a week before tumor implantation. Animal welfare and experimental procedures were carried out strictly in accordance with the “Guide for the Care and Use of Laboratory Animals” (National Research Council, 1996). The xenografts tumor models were established by subcutaneously injecting A375 cells (2×10^6) into the right flank of mice. When tumors reached 100–150 mm³, mice were divided randomly into four groups (8 mice/group) and were treated by intratumoral injections of ZD55-IL-24 (5×10^8 PFU/dose per day) with three consecutive

daily or treated with PBS as a control. For the combination group, the animals were treated locally with a single application of radiation (10 Gy) on the right thigh using a clinical linear accelerator (Varian Co., Milpitas, CA, USA). The tumor was monitored every week by measuring tumor size using caliper for 30 days. The tumor volume was calculated by the following formula: $V \text{ (mm}^3\text{)} = \text{length} \times \text{width}^2 \times 1/2$. At the end of the experiment, tumors were harvested for additional analyses as described below. Differences in tumor growth were tested for statistical significance.

2.7. Immunohistochemistry staining

Tumors were harvested and fixed in 10% formalin, embedded in paraffin and cut in 4 mm sections. Deparaffinized tumor sections were treated with 3% H₂O₂ for 10 min to block the endogenous peroxidase and incubated with blocking serum (goat serum) at room temperature for 30 min. Immunohistochemistry was carried out with anti-Bax antibody or anti-Bcl-

Figure 5 – Anti-tumor activity of ZD55-IL-24 plus radiotherapy in the A375 xenograft model. Tumors were established by injecting A375 cells subcutaneously into the right flank of nude mice. When the tumor reached 100–150 mm³, mice were divided randomly into four groups (8 mice/group) and were received irradiation (10 Gy/d for three consecutive days), ZD55-IL-24 (5×10^8 PFU/d for three consecutive days), or a combination of both. The tumor size was measured and tumor volume was calculated. Data are expressed as means of tumor volume $\pm$ SD ($n = 6$).

2. After incubation with an anti-mouse secondary antibody, the expressions of Bax or Bcl-2 were detected with diaminobenzidine (DAB; Sigma, St. Louis, MO) by enhancement with an avidin–biotin reaction ABC kit (Vector Laboratories Burlingame, CA). Tissue sections stained without primary antibody served as negative control. The slides were then counterstained with hematoxylin. For evaluation of Bax and Bcl-2-positive fractions, at least 200 cells were counted from six different regions and the mean number was determined.

2.8. TUNEL analysis

Apoptotic cells in tumor tissue sections were quantified using in situ apoptosis detection kit (Roche, Indianapolis, IN). Formalin-fixed paraffin-embedded sections were dewaxed before being permeabilized with proteinase K for 15 min at room temperature. Endogenous peroxidase was blocked with 3% H₂O₂, and sections were incubated with equilibration buffer and terminal deoxynucleotidyl transferase (TdT) enzyme. Finally, the sections were incubated with antidigoxigenin-peroxidase conjugate. Peroxidase activity in each tissue section was shown by the application of DAB. Under microscopy, six fields were randomly selected from every sample and 100 cells were randomly selected from every field. The apoptotic rate = (number of total apoptotic cells/100) $\times$ 100%.

2.9. Statistical analysis

Data were expressed as the mean $\pm$ SD, and were analyzed using independent samples t-test and variance (ANOVA) as appropriate by statistical software (SPSS Base 13.0 for Windows, SPSS Inc., Chicago, IL, USA). p -values < 0.05 were considered as statistically significant.

3. Results

3.1. ZD55 mediates efficient delivery of IL-24 in A375 melanoma cells

We first examined the efficiency of ZD55-mediated delivery of IL-24 in A375 cells treated with ZD55-IL-24, radiotherapy, or a combination of both. PBS mock-treated cells were used as controls. IL-24 protein levels were determined by immunoblotting. After treatment for 48 h, IL-24 was stably overexpressed in A375 cells and no discernible difference of IL-24 expression was observed in cells treated with either ZD55-IL-24 alone or ZD55-IL-24 plus radiotherapy (Figure 1), demonstrating that ZD55 can mediate a high and stable expression of IL-24, and that radiotherapy does not affect IL-24 expression. Furthermore, we detected similar expression of the adenoviral E1A protein in ZD55-IL-24 infected cells with or without radiotherapy (Figure 1), indicating that ZD55-IL-24 replicates productively in melanoma cells and that radiotherapy does not attenuate the replication ability of the virus.

3.2. ZD55-IL-24 combined with radiotherapy results in enhanced loss of cell viability

To investigate whether ZD55-IL-24 in combination with radiotherapy inhibits the proliferation of melanoma cells, A375 cells were plated in 96-well plates and treated alone with different dosages of radiotherapy (2, 4 or 8 Gy), ZD55-IL-24 alone (0.1, 1 or 10 MOI) or ZD55-IL-24 plus radiotherapy (0.1 MOI + 2 Gy, 1 MOI + 4 Gy, 10 MOI + 8 Gy). Four days following treatment, cell viability was determined by MTT assay. As shown in Figure 2, the combinatorial treatments led to enhanced inhibition of A375 cell proliferation in a dose-dependent manner. In addition, for each concentration tested, treatments with ZD55-IL-24 plus radiotherapy resulted in higher inhibition than that of ZD55-IL-24 or radiotherapy treatment alone ($p < 0.05$).

3.3. Combination of ZD55-IL-24 with radiotherapy down-regulates Bcl-2 and up-regulates Bax in A375 melanoma cells

To elucidate the mechanism underlying apoptosis observed in combination treatment, we monitored the protein levels of Bcl-2, Bax and caspase-3 by immunoblotting after treatment of A375 cells with ZD55-IL-24, radiotherapy, or a combination of both. We observed that Bcl-2 level was decreased in ZD55-IL-24 treatment alone, but was further reduced in combination with radiotherapy (Figure 3). In addition, either PBS or radiotherapy alone did not significantly increase Bax expression, whereas ZD55-IL-24 or ZD55-IL-24 plus radiotherapy significantly increased Bax expression. Accordingly, procaspase-3 was significantly decreased in the combined treatment (Figure 3). These results suggested that ZD55-IL-24 plus radiotherapy enhanced apoptosis in melanoma cells by efficiently tilting the balance of Bcl-2 family proteins toward the pro-apoptotic pathway.

Figure 6 – The expression of Bcl-2 and Bax in the A375 xenograft model was assessed by immunohistochemistry analysis. (A) Representative photomicrographs showing the Bcl-2 and Bax expression. (B) ZD55-IL-24 plus radiotherapy significantly reduced the number of Bcl-2-positive cells and induced the number of Bax-positive cells (Original magnification $\times 400$) ($n = 6$).

3.4. Combination of ZD55-IL-24 with radiotherapy enhances the activation of the mitochondria-mediated apoptosis pathway in A375 melanoma cells

To verify that the therapeutic effects were due to the inhibition of Bcl-2 and the induction of Bax, A375 cells were frozen and subjected to immunohistochemical analysis of Bcl-2 and Bax. In comparison to the radiotherapy and PBS-treated groups, ZD55-IL-24 and ZD55-IL-24 plus radiotherapy treated groups showed a significant increase in Bax-positive cells. Similarly, ZD55-IL-24 plus radiotherapy clearly demonstrated decreased Bcl-2-positive cells compared to ZD55-IL-24 treatment or radiotherapy (Figure 4). These results indicated the enhanced activation of the mitochondria-mediated apoptosis pathway by combination treatment of ZD55-IL-24 with radiotherapy.

3.5. Anti-tumor efficacy of ZD55-IL-24 plus radiotherapy in nude mice

Based on the *in vitro* data above, we investigated the effects of ZD55-IL-24 plus radiotherapy treatment on tumor growth

in vivo. Nude mice received 2×10^6 A375 cells subcutaneously. When the tumor reached 100–150 mm³, mice were divided randomly into four groups (8 mice/group) and were received irradiation (10 Gy/d for three consecutive days), ZD55-IL-24 (5×10^8 PFU/d for three consecutive days), or a combination of both ($n = 6$). A group of mice receiving A375 cells and then PBS injection was included as the control. Nude mice were sacrificed at 30 days after tumor cell inoculation. The tumors were removed for additional analyses as described below. In the control group receiving PBS, tumors displayed rapid and continued outgrowth during the course of the experiment. The mean tumor size was 12,348.3 mm³. The mean tumor size of the radiation treatment group was 2405.3 mm³ and ZD55-IL-24 treatment group was 5971.7 mm³. The mean tumor size of ZD55-IL-24 plus radiation treatment group was 935.8 mm³, which is much smaller than that of the ZD55-IL-24 treatment group ($p < 0.05$) and the radiation treatment group ($p < 0.05$) (Figure 5).

To verify whether the therapeutic effects are due to the inhibition of Bcl-2 and the induction of Bax, the tumor tissues were frozen and used for the determination of Bcl-2 and Bax expression using immunohistochemical staining. Both ZD55-IL-24 and radiotherapy increased Bax expression. A combination

Figure 7 – Induction of apoptosis after treatment with ZD55-IL-24 plus radiotherapy in the A375 xenograft model. Tumor sections were excised and analyzed for apoptosis by TUNEL staining. (A) Representative photomicrographs showing TUNEL staining in the implanted tumor. (B) Quantitative representation of the proportion of tumor cells manifesting apoptotic changes (Original magnification $\times 400$) ($n = 6$).

of ZD55-IL-24 and radiotherapy further increased Bax expression. ZD55-IL-24 plus radiotherapy suppressed Bcl-2 expression more potently than ZD55-IL-24 treatment or radiotherapy alone (Figure 6). Apoptotic cells in tumor sections were analyzed by TUNEL staining. The apoptotic rate in the ZD55-IL-24 treatment group was $(32.60 \pm 1.89)\%$, the radiation treatment group was $(38.12 \pm 4.47)\%$, and the control PBS group was $(6.23 \pm 2.62)\%$. TUNEL staining showed marked higher apoptotic rate in the ZD55-IL-24 plus radiotherapy group $(84.3 \pm 3.20)\%$, which was significant than the other two treatment groups (Figure 7).

4. Discussion

Melanoma is an extremely aggressive and fatal tumor. Currently, there are no effective treatment modalities and prognosis is poor. Radiotherapy is one of the first line of treatment for melanoma, but the severe side effects of radiotherapy and tumor radioresistance greatly reduce its therapeutic efficacy (Su et al., 2006). Therefore, there is an urgent need to develop effective new therapeutic strategies which decrease tumor recurrence and distant metastasis. Recently, Weichselbaum et al. (Datta et al., 1992; Weichselbaum et al., 1992) reported a new therapeutic strategy of gene radiotherapy which took advantage of the dual anti-tumor effects of gene therapy and

radiotherapy: a certain exogenous gene was chosen that could be activated by radiation, followed by the transcription of cytotoxic proteins to amplify the anti-tumor effect.

IL-24 is a good candidate for sensitization of tumor cells to radiotherapy without exacerbating toxicity, because of its tumor specific anti-angiogenic, pro-apoptotic, and growth-inhibitory activities (Chada et al., 2006; Emdad et al., 2006; Su et al., 2006). A synergistic efficacy profile was observed in the combination of a replication-defective adenovirus expressing IL-24 (Ad-IL-24) with radiation in animals with intracranial primary human GBM tumors (Yacoub et al., 2008). Moreover, a recent study showed that Ad-IL-24 radiosensitized non-small cell lung cancer (NSCLC) cells by enhancing the apoptotic pathway (Nishikawa et al., 2004). However, due to the replication defect of these adenoviral vectors, relatively low amount of IL-24 can be transduced into tumor cells. Such vector systems have not yet demonstrated an improved efficacy especially for the treatment of large solid tumors (Jiang et al., 2010; Yoon et al., 2006).

In contrast to conventional vectors that do not discriminate between tumor and normal cells, CRAds selectively replicate in tumor cells (Jiang et al., 2011). In addition, CRAds could deliver and amplify therapeutic genes in target tumor cells (Jiang et al., 2011). Studies in animal models demonstrated that radiation could enhance the anti-tumor efficacy of CRAds. Furthermore, the radiation-enhanced efficacy of

CRAbs mediated therapy may also result from increased viral replication owing to ionizing radiation (Bieler et al., 2008; Chu et al., 2004; Freytag et al., 2007). Given the independent mechanism of oncolytic virus and ionizing radiation, cross-resistance is theoretically unlikely, thereby minimizing the possibility of development of treatment-resistant tumor cells (Bieler et al., 2008; Chu et al., 2004; Freytag et al., 2007).

In this report, we examined the efficacy and toxicity of combining ZD55-IL-24-mediated gene therapy with radiation therapy in melanoma cells. Western blot analysis confirmed the higher expression of E1A protein in cells treated with either ZD55-IL-24 alone or ZD55-IL-24 plus radiotherapy. We observed elevated expression of transgenic IL-24 only in ZD55-IL-24 plus radiotherapy or ZD55-IL-24-treated melanoma cells. These results indicated that ZD55-IL-24 could efficiently replicate in melanoma cells, and that radiotherapy did not affect the expression of IL-24. Consistent with the ability to replicate in tumor cells, the MTT assay showed that ZD55-IL-24 plus radiotherapy could specifically induce cytopathic effects in melanoma cells. Significant tumor growth inhibition was also demonstrated in the ZD55-IL-24 plus radiotherapy treatment groups when compared with the ZD55-IL-24 or radiotherapy.

It is well established in multiple model systems that the relative levels of the gene products protecting cells from apoptosis (anti-apoptotic proteins, such as Bcl-2 or Bcl-xl) and those promoting apoptosis (pro-apoptotic proteins, such as Bax) are important arbiters of cell survival or death (Eberle et al., 2008; Lesinski et al., 2008). To further elucidate the mechanism of the radiotherapy and ZD55-IL-24 induced cell death, we determined the effect of ZD55-IL-24, with and without radiotherapy, on the levels of Bcl-2, Bax, and Caspase-3 proteins by Western blotting in melanoma cells. We found that ZD55-IL-24 in combination with radiotherapy dramatically decreased Bcl-2 levels, significantly increased Bax, and induced a much stronger activation of Caspase-3. Immunohistochemical staining in vitro and in vivo also showed that the anti-tumor effect of ZD55-IL-24 plus radiotherapy was correlated with Bax upregulation and Bcl-2 down-regulation. These results suggested that ZD55-IL-24 combined with radiotherapy could induce changes in the levels and ratio of pro-apoptotic to anti-apoptotic proteins in melanoma cells. These changes may contribute to the ZD55-IL-24 plus radiotherapy induced apoptosis.

Several hypotheses have been proposed for the apparent augmented therapeutic effect of combining CRAbs with radiotherapy. Ionizing radiation may improve the induction of apoptosis, leading to better tumor interstitial viral spread. Apoptosis-mediated degradation of tumor tissues could lead to a favorable environment, with voided spaces promoting viral spread by the process of diffusion (Chu et al., 2004; Hakkarainen et al., 2009). The adenovirus E1A gene is a potent inducer of chemosensitivity and radiosensitivity through p53-dependent and independent mechanisms. Our Western blot findings using an antibody detecting adenoviral E1A protein in tumor tissues already clearly revealed that viral spreading was substantially increased when tumors were treated with a combination of ZD55-IL-24 and radiation. The E1A protein present in CRAbs may play a role as a potent inducer that correlates with decreased anti-apoptotic Bcl-2 expression and

pro-apoptotic Bax upregulation, ultimately increasing the rate of apoptosis caused by radiotherapy.

Taken together, our results demonstrated that ZD55-IL-24-mediated gene therapy, in combination with radiotherapy, may provide a novel and effective approach for the treatment of melanoma.

Conflicts of interest

The authors declare no conflict of interest.

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