

Minireview

Targeting Gene-Virotherapy for Cancer

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Abstract Gene therapy and viral therapy for cancer have therapeutic effects, but there has been no significant breakthrough in these two forms of therapy. Therefore, a new strategy called “targeting gene-virotherapy”, which combines the advantages of gene therapy and viral therapy, has been formulated. This new therapy has stronger antitumor effects than either gene therapy or viral therapy. A tumor-specific replicative adenovirus vector ZD55 (E1B55KD deleted Adv.) was constructed and various single therapeutic genes were inserted into ZD55 to form ZD55-gene. These are the targeting gene-virotherapy genes. But experiments showed that a single gene was not effective in eliminating the tumor mass, and therefore two genes were separately inserted into ZD55. This strategy is called “targeting dual gene-virotherapy” (with PCT patent). Better results were obtained with this strategy, and all the xenograft tumor masses were completely eliminated in all mice when two suitable genes producing a synergetic or compensative effect were chosen. Twenty-six papers on these strategies have been published by researchers in our laboratory. Furthermore, an adenoviral vector with two targeting promoters harboring two antitumor genes has been constructed for cancer therapy. Promising results have been obtained with this adenoviral vector and another patent has been applied for. This antitumor strategy can be used to kill tumor cells completely with minimum damage to normal cells.

Key words cancer; therapy; gene; targeting viral vector; targeting gene-virus

Malignant tumors are becoming more and more common and they pose a significant threat to human lives. There are conventional means to treat malignant tumors, such as surgery, chemotherapy and radiotherapy. Unfortunately, these therapies are ineffective for patients suffering from tumors in an advanced stage, so it is necessary to develop a new strategy, such as gene therapy, for the prevention and treatment of cancer. Gene therapy is a method of delivering therapeutic genes into patients. In 1990, Anderson was the first to treat SCID with gene therapy by delivering the ADA gene into patients. Thereafter, the conception of gene therapy has developed rapidly, and is now used extensively in the treatment of tumors. More than 1000 protocols of gene therapy have been established, 63% of which are for cancer gene therapy. Adenoviruses are widely used in tumor gene therapy. Although there

were great expectations of tumor gene therapy, there has been no clinical breakthrough so far. Therefore, viral therapy for cancer is becoming increasingly popular again [2–4], involving such adenoviruses as ONYX-015, CV706, HSVG207, CV787, G1716 and NV1020. Among them, the combination of ONYX-015 and chemotherapy (5-FU and cisplatin) achieved a therapeutic effect of more than 60%. When using ONYX-015 alone, the efficacy is less than 15%. Although the Phase II clinical trial of ONYX-015 has finished, the Phase III clinical trial was stopped soon after the start. So far, there has been no significant breakthrough in viral tumor therapy yet. So we proposed a new strategy called “targeting gene-virotherapy”, which combines the advantages of gene therapy and viral therapy [5]. Basically, the antitumor gene is inserted into the tumor-specific replicative viral vector (also called the oncolytic virus) which then delivers the antitumor gene to the tumor cells (**Fig. 1**). The antitumor gene was found

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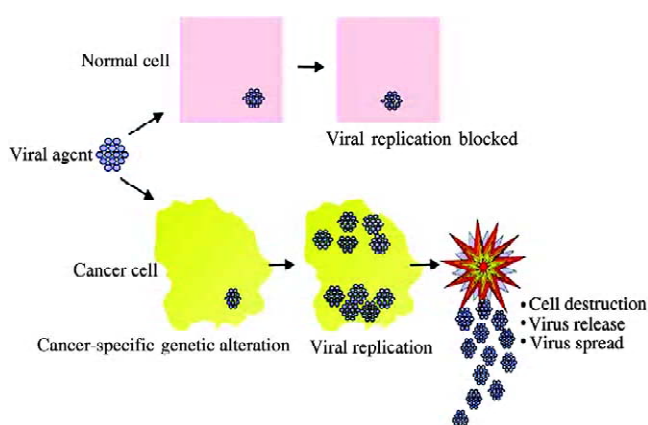


Fig. 1 Targeting antitumor effect of the oncolytic virus

The upper part shows a normal cell, in which the virus cannot replicate. The lower part shows a cancer cell, in which the virus replicates quickly, killing the tumor and releasing new viruses to infect tumor cells in the vicinity.

to be expressed several hundred- to several thousand-fold in tumor cells through the conditional replication of the virus vector. Gene-virotherapy overcomes the shortcomings of conventional tumor gene therapy by using the replication deficiency adenovirus, which results in a low transduction efficiency, low targeting, low expression of antitumor gene and finally, low killing effects. Therefore, our strategy invariably has a higher therapeutic efficacy than conventional strategies.

Construction of Tumor-conditional Replicative Viral Vector

Viral vectors are divided into two types. One type is able to integrate into the chromosome, such as the retrovirus, adeno-associated virus (AAV) and lentivirus. The other type of viral vector, such as the adenovirus, EB virus and HSV, is unable to integrate into the chromosome and stays outside the DNA genome. We have been conducting research into the adenovirus for years, as well as the AAV.

There are many ways to construct tumor-specific replicative viral vectors. One way is to delete the genes necessary for viral replication in normal cells, but which are unnecessary for tumor cells, such as ONYX-015 [1,6]. E1B55KD is an early protein of the adenovirus, which binds to p53 and inhibits its function [7]. p53 is the main anti-adenovirus protein in the host cells. p53 is immediately activated after adenovirus infection and then the infected cells are induced to undergo apoptosis [8], which prevents

the virus from spreading to neighboring tissues. However, E1B55KD is not required for virus replication in p53-deficient tumor cells as there is no p53 to be activated. Thus, the E1B55KD-depleted adenovirus can not replicate in normal cells, but replicate in large amounts in p53-deficient tumor cells. The replicated adenovirus lyses the tumor cells, releases more viruses to infect other tumor cells, and finally eradicates the tumor [6]. We constructed the modified adenoviral vector pZD55, with E1B55KD deleted, but E1A remained, which specifically targeted p53-deficient tumor cells. The recombinant adenovirus was named ZD55, and it is similar to ONYX-015, but it is Ad5, not Ad2/Ad5 fusion virus, and furthermore there is a cloning site in ZD55 which is convenient to insert an exogenous gene into it [9].

Another way to construct tumor-specific replicative viral vectors is to introduce a tumor-specific promoter to control the essential elements of viral replication, such as E1A and E1B. Therefore, these viruses and the inserted antitumor genes can only be replicated and expressed in tumor cells [10]. So it is rational to expect that the antitumor gene products specifically target and kill the tumor cells. Many tissue-specific promoters are available for constructing tumor cell-specific replicative adenoviruses, such as AFP promoter for hepatic cancer, PSA promoter for prostate cancer, and MUC-1 promoter for breast cancer. Certain therapeutic effects have been observed in the use of tumor-specific replicative viral vectors [11–14] (Table 1).

Recent studies have shown that telomerase plays an important role in cell transformation, tumorigenesis, eternalization and development [15,16]. Human telomerase reverse transcriptase (hTERT) is the core component of telomerase and is the most extensively used tumor marker as well. hTERT is not physiologically active in normal cells, but is highly active in 85%–90% of human tumor cells. Moreover, the activity of hTERT is correlated with

Table 1 List of cancer-specific targeting promoters (or elements)

Promoter type	Genes
Targeting all tumors	TERT, HIF-1 (hypoxia promoter), HRE (HIF-1 binding element), E2F (S-phase of cell cycle)
Targeting individual cancers	AFP (liver), CEA (stomach, colorectal), DF3/MUC-1 (breast), tyrosinase (melanoma), SPB (lung)
Targeting specific tissues	PSA (prostate), TTR (liver), albumin (liver)

malignancy, which implies that hTERT is a very good target for cancer therapy. hTERT expression is highly correlated with telomerase activity and is the critical factor regulating telomerase activity in cells [17,18]. hTRER expression is mainly regulated at the transcription level and is up-regulated in 85%–90% of cancer cells, but not transcribed in resting cells [19]. The hTERT promoter has been cloned by several researchers [19,20] and was utilized to drive exogenous gene expression [21,22]. It can also restrain the exogenous gene expression in cancer cells. We replaced the E1A promoter with the hTERT promoter and constructed a novel tumor-specific replicative adenovirus named Ad hTERT [23]. Our study showed that Ad hTERT specifically expressed E1A in tumor cells, but not in normal cells, and that the Ad hTERT virus replicated in tumor cells and then lysed the cells. Animal tumor model data have indicated that Ad hTERT significantly inhibits tumor growth in at least two different forms of cancer [23]. This is the first report of the tumor-specific replicative virus controlled by the hTERT promoter, based on its high activity in tumor cells. The Ad hTERT that we constructed is a highly efficient and general tumor cell-killing agent, and the application of the hTERT promoter in the regulation of E1A and E1B expression provides a basis for tumor therapy. The therapy using Ad hTERT mentioned above is still a form of virotherapy. When an antitumor gene is inserted into Ad hTERT, this therapy becomes a gene-virotherapy [24,29].

Targeting Gene-Virotherapy

Targeting gene-virotherapy has two advantages. The first advantage is that the viral vector can replicate specifically in tumor cells and kill them accordingly. After inserting the antitumor genes into ZD55 to form ZD55-genes, these genes will specifically replicate and kill tumor cells at a rate 600–1000 folds higher than that in normal cells. The second advantage is that exogenous gene expression increases several hundred- to several thousand folds in parallel with viral replication. Our data revealed that gene expression is time-dependent and increases with infection.

Different gene-virotherapy genes can be created using ZD55 by inserting different antitumor genes. The following antitumor genes are available: (1) the tumor necrosis factor-related apoptosis-inducing ligand (Trail) pro-apoptotic gene, which induces apoptosis in tumor cells but not in normal cells; (2) Smac (the second mitochondria-derived activator of caspase) gene, which blocks the effect of IAP, inhibitor of apoptosis protein; (3) tumor suppressor genes, such as p53, Rb, PTEN, LFIRE, LPTS, etc.; (4)

immune regulator genes, such as IL-2, IL-12 and IL-24; (5) angiogenesis inhibitory genes, such as angiostatin, k5, endostatin, VEGI and sFlt1; (6) suicide genes, such as CD and TK; and (7) anti-free radical genes, such as MnSOD, etc. (**Table 2**). We have inserted many of the above antitumor genes into ZD55 to form ZD55-gene, respectively. The therapeutic effect of this targeting gene-virotherapy is much higher than ONYX-015 or ZD55 alone. The results of our research have been published in many journals [25,27,30].

Table 2 List of available therapeutic genes

Therapeutic type	Genes
Tumor suppressor gene	p53, Rb, LFIRE, LPTS, Cyld, PTEN, MnSOD
Apoptosis-inducing gene	Trail, Smac, IL-24, caspase 3
Immune regulator gene	GM-CSF, IL-2, IL-12, CD40L, MIP-3a, IP-10
Angiogenesis inhibitor gene	k5, PEDF, SFLK1, sFlt-1, VEGI, endostatin, angiostatin
Suicide gene	TK, CD
IAP gene	X-IAP, CIAP1, CIAP2

Our data on ZD55-IL-24 [**Fig. 2(A,B)**] have been published in *Human Gene Therapy* [30] and the pharmacological and toxicological study of ZD55-IL-24 will start soon. The reason of choosing this antitumor drug for clinical use is that in the USA, the ZD55-like virus ONYX-015 has been found to have little or no side effects in the clinical Phase III trial and Ad-IL-24 has been in the clinical Phase I/II trial. Furthermore, the antitumor effect of ZD55-IL-24 is hundreds folds higher than that of Ad-IL-24.

Targeting Dual Gene-Virotherapy

Targeting gene-virotherapy has been shown to successfully eradicate the transplanted tumor in animal models in some individual cases, but can not eliminate all tumors *in vivo*. In order to improve this targeting gene-virotherapy strategy, we proposed another new strategy, targeting dual gene-virotherapy. In this strategy, two genes are inserted into ZD55 or Ad TERT. The two genes have a synergetic or complementary antitumor effect. It has been found that the application of ZD55-Trail and Ad-k5 together completely

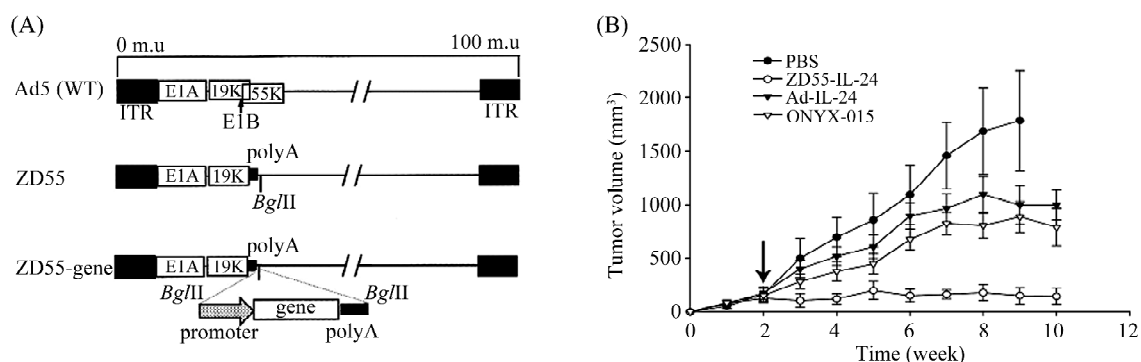


Fig. 2 Targeting gene-virotherapy using interleukin-24 (IL-24)

(A) The first line shows a wild-type adenovirus with 19K and 55K genes in E1B. The second line shows the ZD55 virus with the 55 kDa gene deleted. The third line shows our ZD55-gene in which a cassette harboring the IL-24 gene was inserted. (B) The therapeutic effect of PBS, ZD55-IL-24, Ad-IL-24 and ONYX-015 on tumor volume. ZD55-IL-24 is ready for preclinical pharmacological and toxicological animal studies.

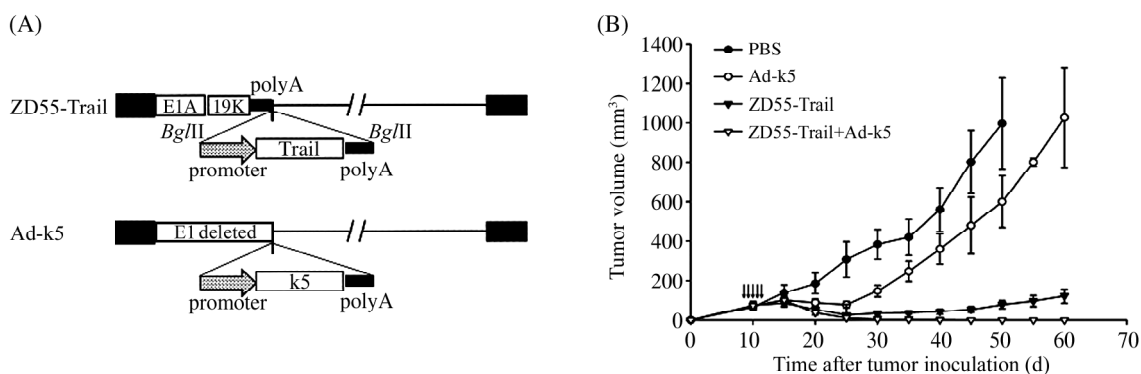


Fig. 3 Targeting dual gene-virotherapy using the combined effect of ZD55-Trail and Ad-k5

(A) The two viruses ZD55-Trail and Ad-k5. (B) Therapeutic effect curves.

eradicates all transplanted colorectal cancers induced by SW620. The antitumor effect of Trail is a result of apoptosis and that of k5 is a result of the anti-angiogenesis of tumor blood vessels, so these two proteins inhibit and kill tumor cells through different mechanisms and have a synergistic effect. The research results have been published in *Molecular Therapy* [Fig. 3(A,B)] [26].

Recently, the antitumor effect of ZD55-MnSOD was found to be about 1000 folds higher than that of Ad-MnSOD. A new function of Trail was discovered whereby it induces the expression of MnSOD by the induction of ZD55-Trail. Accordingly, by the combined use of ZD55-Trail and ZD55-MnSOD, all colorectal xenograft tumors were completely eliminated in a previous study [Fig. 4 (B)] [31]. Moreover, a new mechanism of H_2O_2 produced by MnSOD has been found to be mediated by caspase 8

[Fig. 4(C)].

IAP is an inhibitor of apoptosis. When the IAP level is high, cells will become cancerous easily. Chemotherapy and Trail treatment are ineffective against high-IAP cancers. BEL-7404 is a hepatic carcinoma cell line with a high level of IAP. This cell line and its xenograft cancer are also unaffected by chemotherapy or Trail. But Smac abolishes IAP activity. By using a combination of ZD55-Trail and ZD55-Smac, it has been shown that all the BEL-7404 cells can be killed *in vitro*, and all the transplanted liver cancers can be completely eradicated *in vivo* (Fig. 5). As Smac can block the action of IAP and Trail can induce Smac, they have a synergistic effect. This strategy can also be used as a basis for the treatment of cancers with a high IAP level [28].

In summary, targeting dual gene-virotherapy is a very

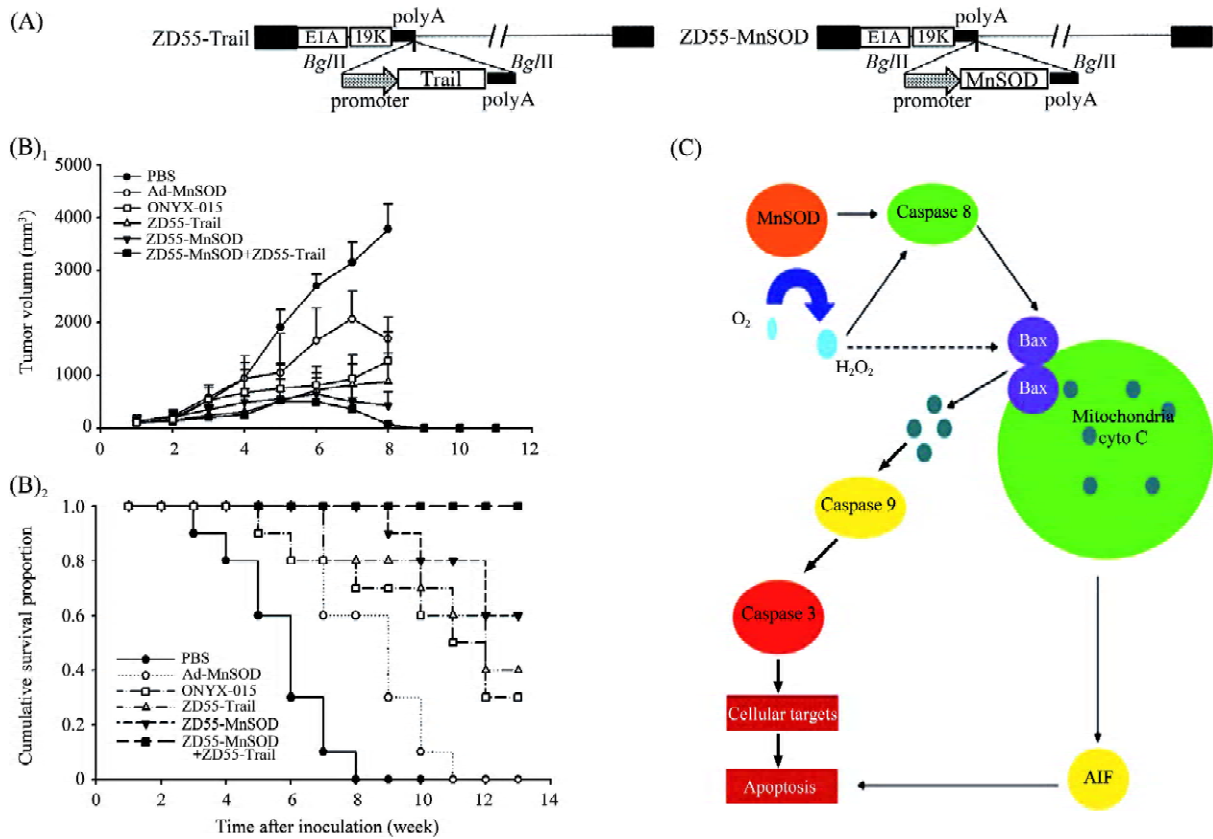


Fig. 4 Combined therapeutic effect of ZD55-Trail and ZD55-MnSOD and their killing effects

(A) A construction of ZD55-Trail and ZD55-MnSOD. (B) Combined therapeutic effect of ZD55-Trail and ZD55-MnSOD. (C) The signal pathway of hydrogen peroxide is mediated by caspase 8 (solid line) rather than the direct induction of Bax (dotted line).

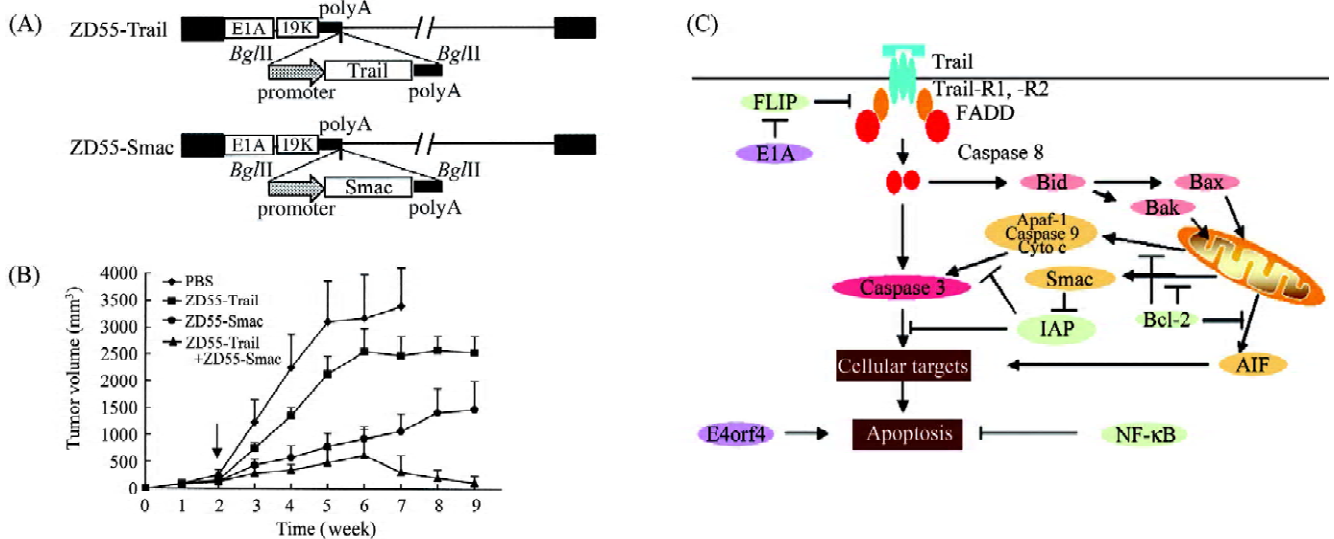


Fig. 5 Basis for the treatment of high-IAP cancer

(A) The two genes Trail and Smac with synergetic effect were used. (B) Therapeutic effect of combined ZD55-Trail and ZD55-Smac (lowest curve). (C) This shows how IAP blocks the key apoptosis molecule (caspase 3) and how Smac blocks the action of IAP. Smac can increase the killing effect of Trail through the action of caspase 3. In addition, Trail can induce the release of Smac. Therefore, the two genes, Trail and Smac, have a synergetic effect.

good strategy for tumor biotherapy and has won the Debiopharm-CCRF award in China.

Double-Targeting Virus Double-Gene Therapy

An ideal tumor-targeting vector should be able to specifically target all tumor cells with minimum toxicity to normal cells. ZD55 and Ad hTERT (E1A was controlled by hTERT) can essentially satisfy the above requirement, but they are not selective enough as replicative viruses. Therefore, the targeting effect should be further enhanced and the double-targeting viral vector was constructed using two promoters (hTERT, HRE, HIF-1, AFP, CEA, PSA, etc.) to regulate virus replication. Accordingly, these viral vectors, which harbor one or two genes, can target tumor cells more specifically and achieve a better tumor-killing effect. Therefore, a strategy called double-targeting virus double-gene therapy was formulated using two different genes and two different targeting promoters. TERT-HRE-Trail/Smac, hTERT-AFP-Trail/Smac, TERT-ZD55-Trail/K5, etc. were constructed. We expect this strategy to have excellent antitumor effects in the clinical trial.

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