

The Excellent Antitumor Effect of IL-24 and the Complete Eradication of the Xenograft Tumor with CTGVT-DG Strategy

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Abstract

This paper is to emphasize the higher antitumor effect of IL-24, because it can make the complete elimination of the xenograft tumor. Another important point in this paper is to introduce a strategy for essentially complete eradication all the xenograft tumor which was named "Cancer Targeting Double Gene-Viro-Therapy or the Cancer Targeting Gene-Viro-Therapy with double gene (CTGVT-DG)".

Keywords: Cancer; Apoptosis; Gene therapy; Tumor

Introduction

A novel gene mda-7 (melanoma differentiation-associated gene 7), was identified by subtraction hybridization using a human melanoma cell line (HO-1) [1-5]. Because of structure of mda-7 similar to the interleukin 10 (IL-10) family of cytokines with chromosomal localization and cytokine-like properties, mda-7 has been redesignated as IL-24 [2-5]. The mda-7 cDNA encodes a protein of 206 amino acids with a predicted size of 23.8 kDa [6].

Many studies have shown that enforced expression of IL-24 suppresses cell growth and induces apoptosis in a variety of tumor types including melanomas, gliomas, and cancers of the breast, colon, lung, cervix, pancreas, and prostate [7-15]. In contrast, these investigations also demonstrated that elevated expression of mda-7/IL-24 in normal mammary epithelial cells has no cytotoxic effects [16,17]. Studies showed that mda-7/IL-24 induces growth suppression and apoptosis in diverse cancer cells [5,14]. In addition to its direct antitumor activity, mda-7/IL-24 also exerts antiangiogenic activity *in vivo* [18].

Because IL-24 is a novel and prospective gene for the therapy of multiple cancers, understanding the mechanism by which this gene induces apoptosis in cancer cells will be of immense value. Studies are beginning to high light on the signaling cascades involved in mda-7/IL-24 induction of apoptosis [19-22]. Analyses of signaling pathways have revealed Ad-IL-24 regulation of inducible nitric oxide synthase (iNOS) and mitogen-activated protein kinase (MAPK) in melanoma [23] and Jun kinase, h-catenin, phosphatidylinositol 3-kinase (PI3K), and protein kinase R(PKR) in lung and breast tumor cells [24,25]. In the following text, I will describe the excellent antitumor effect of IL-24 on prostate cancer or hepatoma and the complete elimination of the xenograft tumor by the CTGVT-DG strategy.

Study on the Excellent Antitumor Effect of IL-24 on Prostate Cancer

We have made two constructs to study their antitumor effect: The Ad-DD3-E1A-WPRE-E1B (Δ 55)-(PTEN) (Figure 1a) [26] the Ad-IL-24-DD3-E1A (Figure 1b) [27]. In (Figures 1a and 1b), both the DD3, a prostate cancer specific promoter, were used to replace the native promoter in E1A of adenovirus and drive the OncoAd (oncolytic virus from adenovirus) to specific targeting to prostate cancer and used the same CMV promoter to drive the two expression cassettes of PTEN gene and IL-24 gene. Although, PTEN is a brood cancer suppressor gene with rather prostate cancer tropism, but we added a WPRE to

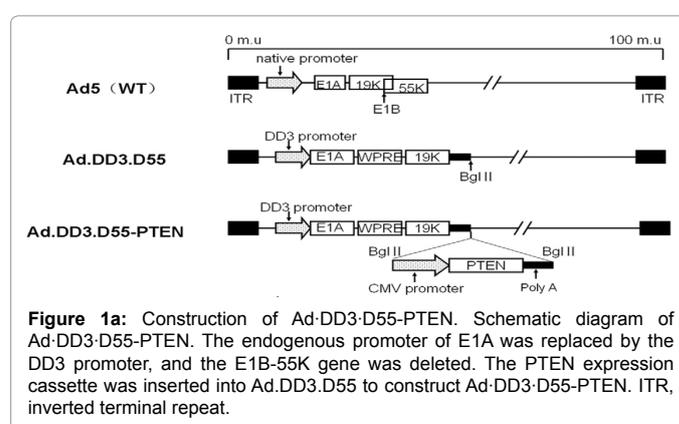


Figure 1a: Construction of Ad-DD3-D55-PTEN. Schematic diagram of Ad-DD3-D55-PTEN. The endogenous promoter of E1A was replaced by the DD3 promoter, and the E1B-55K gene was deleted. The PTEN expression cassette was inserted into Ad-DD3-D55 to construct Ad-DD3-D55-PTEN. ITR, inverted terminal repeat.

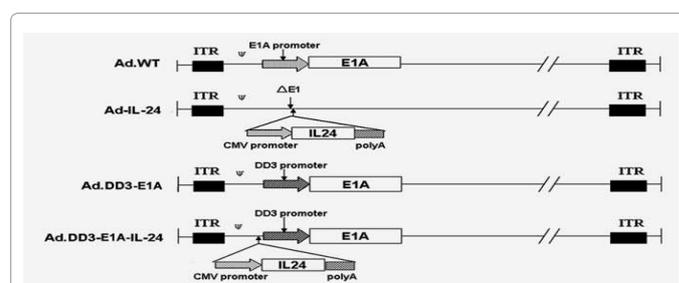


Figure 1b: Construction of Ad-DD3-E1A-IL-24. Schematic diagram of the viruses. In Ad-DD3-E1A, the endogenous promoter of E1A was replaced with the DD3 promoter. The expression cassette of IL-24 was inserted into Ad-DD3-E1A to construct Ad-DD3-E1A-IL-24. Ad-IL-24 is a replication-deficient adenovirus with a deleted E1 region. ITR, inverted terminal repeats; ψ , packaging signal.

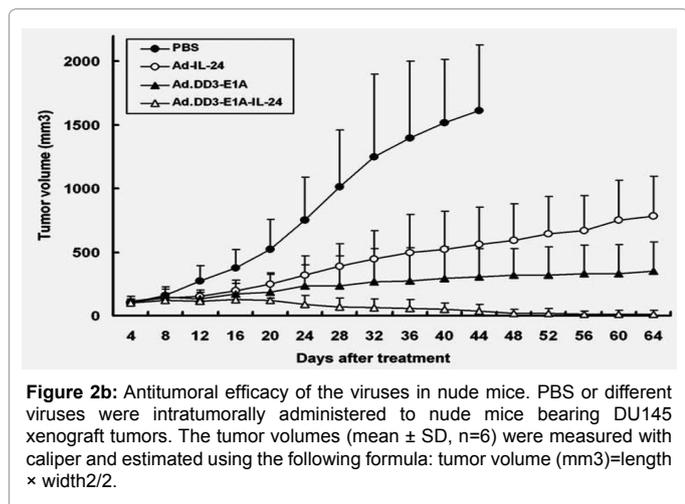
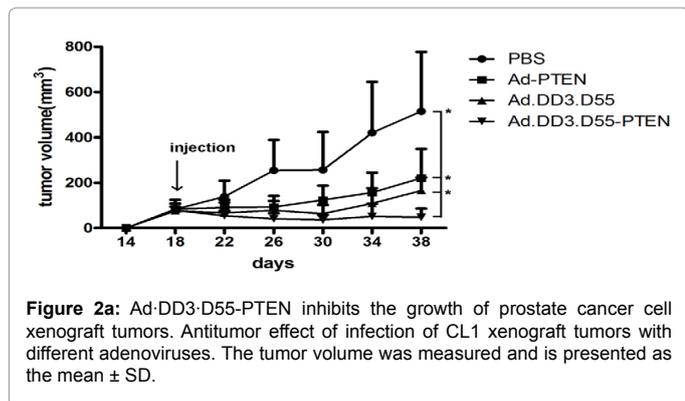
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enhance its activity of mRNA more stable and deletion 55 KD in E1B to make the adenovirus more specific targeting to cancer cells as shown in (Figure 1a). All of these were lack in (Figure 1b) which only the IL-24 was use without any helpless (Figure 1b) [27]. However, the xenograft prostate cancer can be completely eliminated by the only IL-24 gene (Figure 2b) [27]. Which is better than (Figure 2a) [26], showing the excellent antitumor effect of IL-24.



Study on the excellent antitumor effect of IL-24 on hepatoma

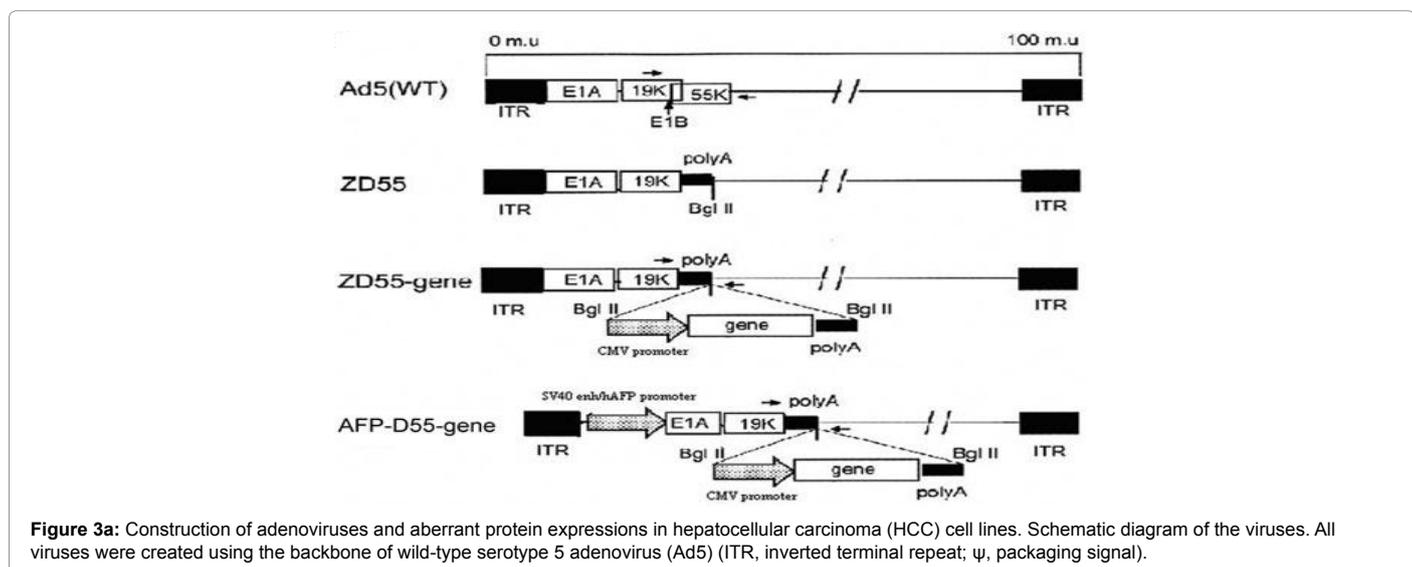
In liver cancer, a specific promoter AFP was replaced the native E1A promoter of adenovirus to make more specific to live cancer and deleted the 55 KD of E1B in adenovirus was deleted to make it more targeting to cancer cell, then we can make two products (Figure 3): 1. Ad-enAFP-E1A-E1B(Δ55)·(Trail) (AFP-D55-Trail) and 2. Ad-enAFP-E1A-E1B(Δ55)·(SOCS3) (AFP-SOCS3-Trail) as shown in (Figure 3a) [28], the Trail is a gene from TNFα superfamily, the TNFSF10, which has good antitumor effect, but much less toxicity comparing with TNFα itself, the SOCS3 is good and rather liver specific tropism antitumor gene.

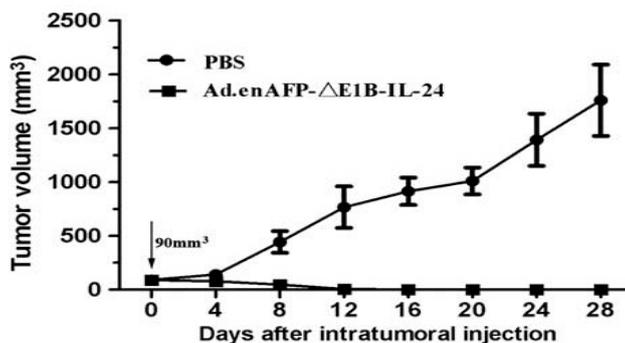
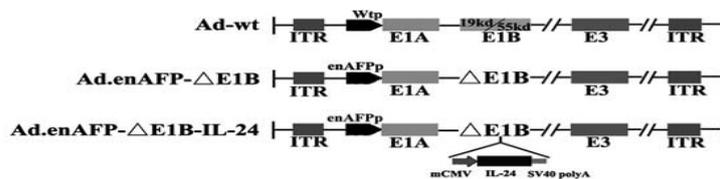
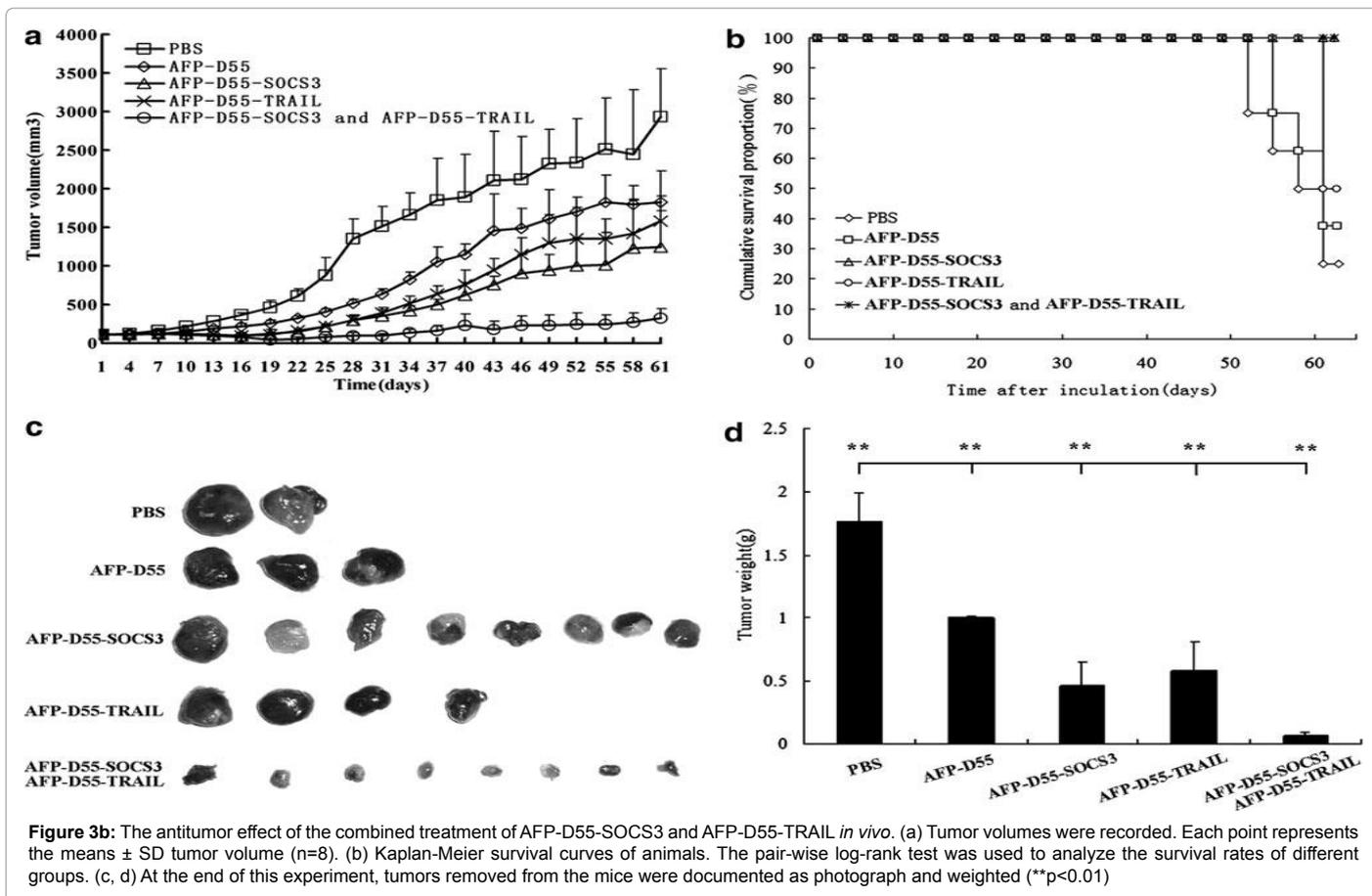
By the combine of constructs 1 and 2, good anti-hepatoma effect was obtained as shown in (Figure 3a) [28]. However, the antitumor effect of two gene, the SOCS3 plus Trail as shown in (Figure 3b) was less than that of only one IL-24 gene construct as shown in (Figures 4a and 4b) [29], showing also the super antitumor effect of IL-24 gene.

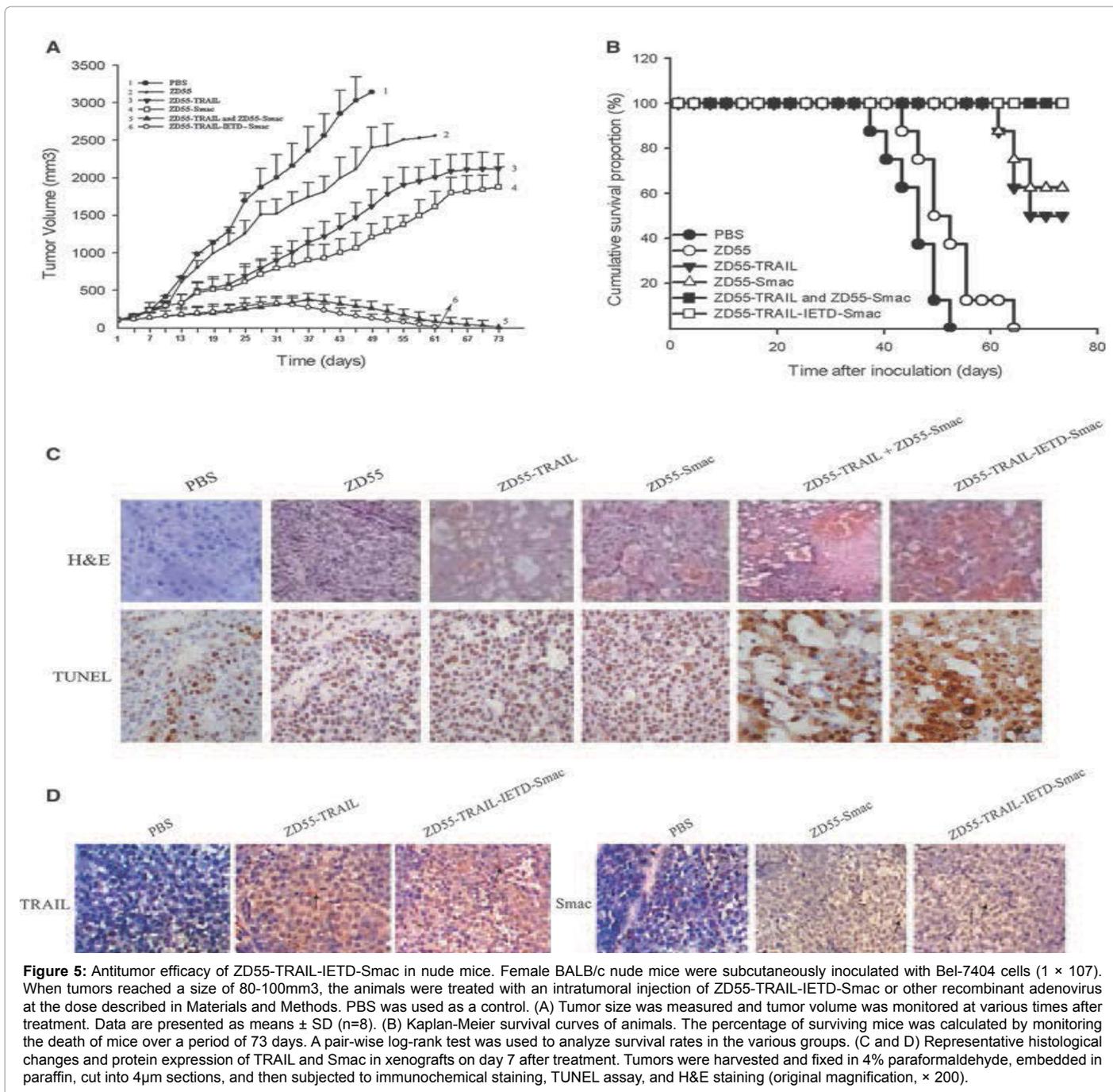
Excellent antitumor effect of the CTGVT-DG strategy

Currently there are cancer gene therapy and cancer oncolytic virotherapy two fields. We innovate a third field, by inserting an antitumor effect into an oncolytic virus (OV-gene) [30,31] and named it as Cancer Targeting Gene-Viro-Therapy, CTGVT. The antitumor effect of CTGVT (OV-gene) was very much increased than its original two therapy. It is because that the gene can be induced to highly replication by its vector OV's replication. Therefore, the antitumor effect CTGVT (OV-gene) was much increased [32]. However, if we use two gene in the CTGVT (OV-gene) system i.e. the OV-gene1 plus OV-gene2 or OV-gene1-gene2 and named as CTGVT-DG, by which all the xenograft tumor can be completely eradicated.

Here we only give an example of our CTGVT-DG strategy, the ZD55-Trail+ZD55-Smac or the ZD55-Trail-IETD-Smac as shown in (Figure 5) [33]. Since the two genes we used may have compensative or synergetic effect between them many other CTGVT-DG to complete eradication of xenograft tumor has been obtained by us [34-38]. The antitumor effect of the CTGVT-DG is much higher than that of the antitumor effect of PD-1 antibody or Amgen's excellent drug OncoHSV-GM-CSF (data which will be published later). That is a great success.







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