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Developing adenoviral vectors encoding therapeutic genes toxic to host cells: Comparing binary and single-inducible vectors expressing truncated E2F-1

Jorge G. Gomez-Gutierrez [a,1], Xiao-Mei Rao [b,1], Aracely Garcia-Garcia [a], Hongying Hao [a], Kelly M. McMasters [a,b,*], H. Sam Zhou [a,b,c,*]

[a] Department of Surgery, University of Louisville School of Medicine, Louisville, KY 40292, USA
[b] James Graham Brown Cancer Center, University of Louisville School of Medicine, Louisville, KY 40292, USA
[c] Department of Microbiology and Immunology, University of Louisville School of Medicine, Louisville, KY 40292, USA

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ABSTRACT

Adenoviral vectors are highly efficient at transferring genes into cells and are broadly used in cancer gene therapy. However, many therapeutic genes are toxic to vector host cells and thus inhibit vector production. The truncated form of E2F-1 (E2Ftr), which lacks the transactivation domain, can significantly induce cancer cell apoptosis, but is also toxic to HEK-293 cells and inhibits adenovirus replication. To overcome this, we have developed binary- and single-vector systems with a modified tetracycline-off inducible promoter to control E2Ftr expression. We compared several vectors and found that the structure of expression cassettes in vectors significantly affects E2Ftr expression. One construct expresses high levels of inducible E2Ftr and efficiently causes apoptotic cancer cell death by activation of caspase-3. The approach developed in this study may be applied in other viral vectors for encoding therapeutic genes that are toxic to their host cells and/or inhibit vector propagation.

Introduction

Adenovirus (Ad) is easy to manipulate *in vitro*, produces high titer stocks, and infects a broad range of mammalian cells. For these reasons, Ad vectors (Adv) are preferred in basic and medical research. For cancer gene therapy, Adv are used to deliver genes or modified versions of genes into tumors. The products of therapeutic genes are generally toxic to cancer cells or cause apoptosis; thus, the gene products may be also toxic to human embryonic kidney HEK-293 cells used for Adv production and prevention of vector construction and amplification.

The transcription factor E2F-1 plays important roles in the activation of expression of genes involved in cell cycle progression and growth. Recent studies by us and others have shown that Adv expressing E2F-1 efficiently induce apoptosis in cancer cells *in vitro* and *in vivo* (Dong et al., 1999, 2002; Elliott et al., 2001; Itoshima et al., 2000; Vorburger et al., 2005). However, the oncogenic property of promoting cell proliferation by wild type (wt) E2F-1, presumably by virtue of its ability to stimulate expression of cell cycle-promoting genes, limits its application in cancer gene therapy. To circumvent this barrier, the truncated forms of E2F-1 (E2Ftr) were generated, which lack the transactivation domain and cell cycle-promoting effects (Bell et al., 2006; Fan and Bertino, 1997). Studies have showed that these mutants are potent inducers of apoptosis but are unable to induce cell cycle progression (Fan and Bertino, 1997; Hsieh et al., 1997; Melillo et al., 1994).

We previously attempted to create an Adv using the *Cytomegalovirus* (*CMV*) promoter to control E2Ftr (aa 1–375) without success despite multiple tries (Xiao-Mei Rao, unpublished results), likely because a high level of E2Ftr is toxic to HEK-293 cells and blocks Ad replication. To overcome this, we applied a tetracycline (Tet)-off inducible expression promoter (Gossen and Bujard, 1992) and developed inducible binary- and single-Adv systems to express E2Ftr (Fig. 1). Our binary-vector system consists of two vectors: an Ad helper virus (Adhv) and an Ad-E2Ftr vector. Adhv expresses Tet-regulated transactivator (tTA), which activates the E2Ftr expression cassette in Ad-E2Ftr. Without the helper vector, E2Ftr expression in Ad-E2Ftr is totally inhibited, so Ad-E2Ftr can be created and amplified in HEK-293 cells. High levels of the apoptotic E2Ftr protein are induced in cells only when Adhv is applied with Ad-E2Ftr.

* Corresponding authors. K.M. McMasters is to be contacted at Department of Surgery, University of Louisville Medical School, Louisville, KY 40292, USA. H.S. Zhou, James Graham Brown Cancer Center, University of Louisville Medical School, Louisville, KY 40202, USA.
E-mail addresses: kmmcma01@louisville.edu (K.M. McMasters), hszhou01@louisville.edu (H.S. Zhou).
[1] Contributed equally to this work.

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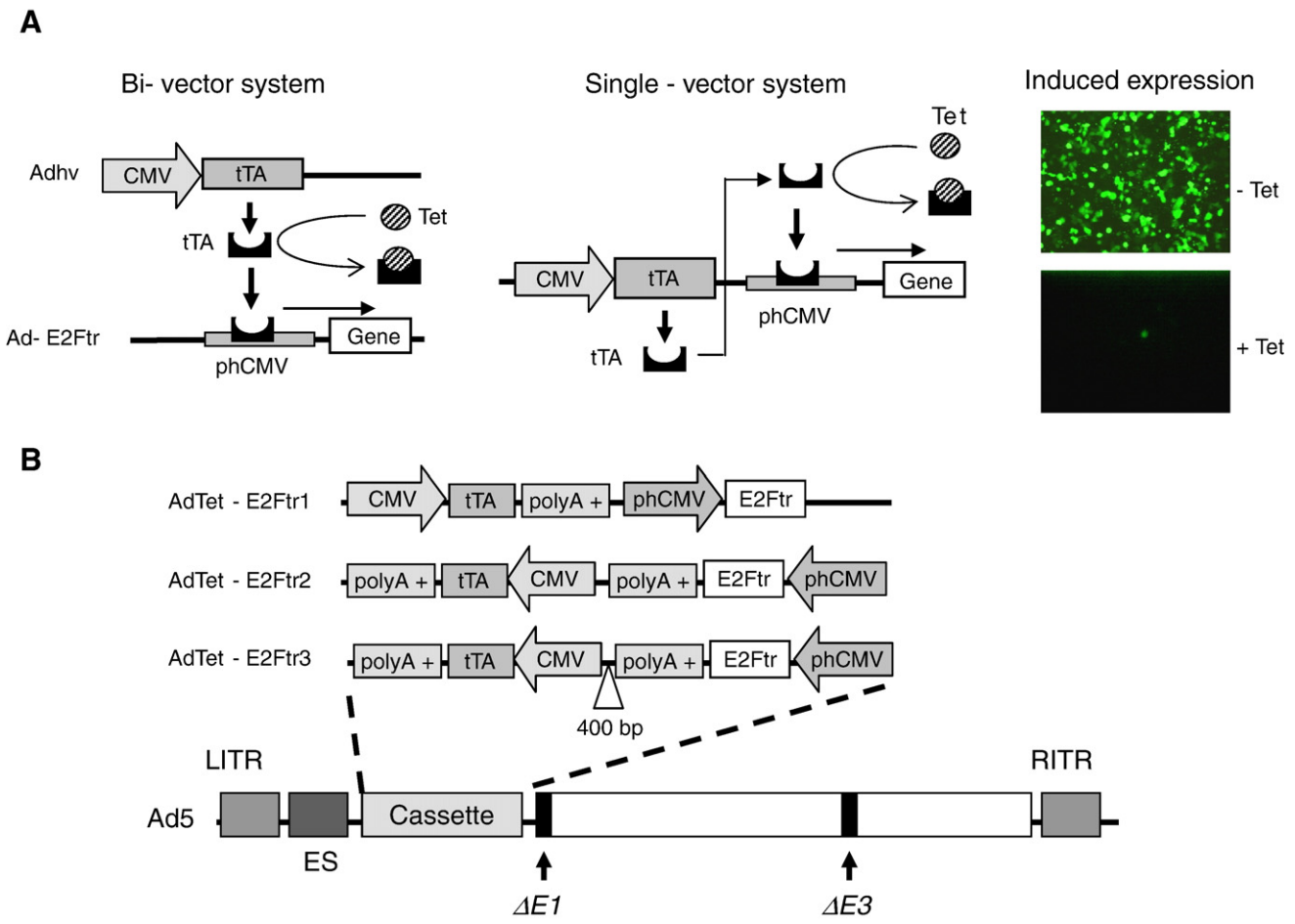

Fig. 1. Schematic diagram of binary- and single-Adv systems. (A) *CMV* promoter drives the expression of the *tTA* protein. *EGFP* or *E2Ftr* is under the control of a synthetic minimal promoter composed of Tet-responsive element (*TRE*) and *CMV* mini-promoter (*phcmv*), which is silent unless activated by tTA. In the absence of Tet or Dox, tTA binds to *phcmv* and triggers the expression of EGFP or E2Ftr. When Tet is added to the medium, tTA is bound by Tet and unable to bind to *phcmv* and activate the expression of EGFP or E2Ftr. SK-MEL-2 cells in the absence or presence of Dox (1 μg/ml) were infected with AdTet-EGFP at an MOI of 50. After 24 h, cells were observed for EGFP expression under fluorescence microscopy and photographs were taken with Kodak MDS 290 software at ×20 magnification. (B) Schema of three single bicistronic Ads encoding E2Ftr, the left and right inverted terminal repeat sequences (*LITR* or *RITR*, respectively); encapsidation signal (ES) and *E1*/*E3* deleted genes are shown in the Ad structure.

Although this system leads to high-level expression of E2Ftr and is useful in research, the absolute need for co-delivery of two separate vectors made the clinical use of this strategy untenable. Thus, we further developed a single-vector system in which both the tTA and E2Ftr expression cassettes are incorporated into one Adv. A helper vector is not required for E2Ftr expression in the single-vector system.

We compared the binary- and single-vector systems expressing inducible E2Ftr. We found that Ad-mediated high-level expression of E2Ftr is toxic to cancer cells as well as to vector host HEK-293 cells. E2Ftr efficiently induces cell death associated with poly (ADP-Ribose) polymerase (PARP) cleavage and procaspase-3 decrease, the hallmarks of apoptosis. Since many other genes which have potential for cancer therapy may be also toxic to vector host cells and inhibit vector production, the approaches reported in this study may have broad applications in creation of viral vectors that will carry toxic cancer therapeutic genes.

Results

Binary- and single-vector systems

Fig. 1A depicts the binary- and single-vector systems with Tet-off inducible transcription cassettes. In the bi-vector system, expression of E2Ftr from Ad-E2Ftr is controlled by the Tet-regulating promoter (phcmv), which is activated by tTA encoded in the helper vector Adhv. In the single-vector system, both transgene and tTA expression cassettes are inserted in the same vector. Expression of the enhanced green fluorescent protein (EGFP) reporter shows the efficient regulation of the Tet-off inducible promoter in the single-vector system (Fig. 1A). Fig. 1B shows the schema of three different single bicistronic Adv encoding E2Ftr under the regulation of the Tet-off system. We did not insert a poly A signal in AdTet-E2Ftr1 because the Ad's backbone can provide the viral E1 gene poly A signal. The tTA and E2Ftr expression cassettes in AdTet-E2Ftr2 are closely connected; while a 400-base pair (bp) fragment from the pcDNA3.1 (+/−) plasmid (Invitrogen, Carlsbad, CA) was inserted between the two cassettes in AdTet-E2Ftr3.

Regulation of E2Ftr expression in binary and single Adv

Human melanoma SK-MEL-2 cells were infected with Ad-E2Ftr alone (control) or co-infected with Adhv in the presence or absence of doxycycline (Dox; 1 μg/ml), a derivative of Tet. At 48 h after infection, E2Ftr expression was analyzed. E2Ftr expression from the binary system was only induced in the presence of Adhv and absence of Dox; adding Dox negatively regulated E2Ftr expression in cells (Fig. 2A).

We further evaluated E2Ftr expression from the three constructs of the single-vector system. SK-MEL-2 cells were infected with AdTet-E2Ftr1, AdTet-E2Ftr2, or AdTet-E2Ftr3 at a multiplicity of infection