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A bidirectional Tet-dependent promotor construct regulating the expression of E1A for tight control of oncolytic adenovirus replication

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Abstract

Tight regulation of oncolytic adenoviruses (oAdV) represents an important requirement for their safe application. Here we describe a new doxycycline (Dox)-dependent oAdV with a bidirectional expression cassette, which drives the expression of the reverse tetracycline-controlled transactivator (rtTA^s-M2) from a lung tumor-specific promoter and, in the opposite direction, the expression of the adenoviral E1A gene from a second generation $TetO_7$ sequence linked to an isolated TATA box. In H441 lung cancer cells, this oAdV showed a strictly Dox-dependent E1A expression, adenoviral replication, cell killing activity and a 450-fold induction of progeny virus production. The virus could be shut off again by withdrawal of Dox and, in contrast to a control oAdV expressing E1A directly from the SP-B promoter, did not replicate in non-target cells. However, the absolute values of virus production and the cell killing activity in the presence of the inducer were still reduced as compared to the control oAdV. The results demonstrate, for the first time, Dox-dependent oAdV replication from a single adenoviral vector genome. Future improvement of the Dox-dependent E1A regulation cassette should lead to the generation of an oAdV well suited to meet the demands for a highly regulated and efficient oncolytic virus for *in vivo* applications.
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Keywords: Tet-On system; Cancer gene therapy; Oncolytic adenovirus

Abbreviations: oAdV, oncolytic adenovirus; Dox, doxycycline; TRE, tetracycline response element; rtTA^s-M2, reverse tetracycline-controlled transactivator; SP-B, surfactant protein B; MOI, multiplicity of infection; Pfu, plaque-forming units

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1. Introduction

Oncolytic adenoviruses (oAdV's) are promising tools for cancer gene therapy and have already been successfully tested in cancer patients or are currently under consideration in clinical trails (McCormick, 2005; Davis and Fang, 2005). To restrict the replication of oAdV's to tumor cells, which is one of the most important requirements for their safe use in humans, a variety of different approaches have been taken. Tumor-specific promoters were used to drive the expression of the adenoviral E1A, E1B or E4 genes (Yu et al., 1999; Doronin et al., 2001; Lukashev et al., 2005; Kuppuswamy et al., 2005). In an alternative approach the E1A/E1B genes were mutated in such a way that their ability to dysregulate cell cycle and pro-apoptotic genes was abolished (Bischoff et al., 1996; Heise et al., 2000). In addition, pharmacologically regulatable oAdV's were developed with the aim of temporally controlling their replication from the outside. Among these are dexamethasone- (Avvakumov and Mymryk, 2002), tamoxifen/4-OH-tamoxifen- (Sipo et al., 2006a), rapamycin- (Chong et al., 2002), and doxycycline (Dox)-dependent oAdV systems (Fechner et al., 2003; Hurtado Picó et al., 2005). An important issue with regard to the development of inducible vectors is the choice of the inducer drug. Dox, an analog of tetracycline, is widely accepted because of its safety when used in humans (Solera et al., 1996). Its high specificity for the bacterial tetracycline repressor (TetR), the low dose of drug needed to induce protein expression (Kringstein et al., 1998), its fast kinetics after addition of the inducer as well as the rapid reversal of induction after its withdrawal (Salucci et al., 2002) make it suitable for clinical use. Therefore, the Tet-regulated system with its two variants Tet-On (Gossen et al., 1995) and Tet-Off (Gossen and Bujard, 1992) has been the most extensively studied. In general, the Tet-On system seems to be better suited for gene therapeutic applications than the Tet-Off system, since it does not require continuous pharmacological treatment after termination of gene expression. It consists of a reverse tetracycline-controlled transactivator (rtTA), which binds to a tetracycline response element (TRE) promoter driving the gene of interest in the presence of Dox only. The original Tet-On system, however, has several limitations. The most serious one is a pronounced leakiness in the absence of the inducer (Gossen et al., 1995). This leakiness was at least partly overcome by the use of second generation rtTAs (rtTA2^s-S2, rtTA2^s-M2) (Urlinger et al., 2000), novel TRE promoters (Agha-Mohammadi et al., 2004; Pluta et al., 2005; Sipo et al., 2006b) or combination of the Tet-On system with a tetracycline controlled transcriptional silencer (tTS) (Freundlieb et al., 1999), which reduces the TRE activity in the absence of the inducer. We have recently developed an oAdV system, which permits Dox-dependent regulation of oAdV replication (Fechner et al., 2003). However, the level of regulation was limited and could only be achieved by the simultaneous application of three different adenoviral vectors: a transactivator vector expressing the rtTA^s-M2, a transsuppressor vector expressing the tTS and the response adenoviral vector expressing the adenoviral E1A gene from the original TRE promoter. To simplify Dox-dependent oAdVs and improve their drug-dependent regulation, we constructed a novel oAdV. It contains a bidirectional, Dox-dependent expression cassette for lung tumor-specific expression of the rtTA^s-M2, which in turn mediates Dox-dependent activation of the adenoviral E1A gene. The vector was thoroughly analyzed for levels of Dox-dependent regulation of E1A expression, for regulation of adenoviral replication, for its oncolytic activities *in vitro* and its specificity for lung-tumor derived target cells as compared to non-target cells.

2. Materials and methods

2.1. Cell lines

The following cell lines were used: HeLa (human cervix carcinoma) cells and HEK293 (human embryonal kidney) cells were cultured in DMEM (Gibco BRL, Karlsruhe, Germany). H441 (human pulmonary adenocarcinoma) cells (Yan et al., 1995) (kindly supplied by J.A. Whitsett, Children's Hospital Medical Center, Cincinnati, OH, USA) were cultured in RPMI 1640 medium supplemented with 2 mM glutamine. EA.hy926 cells (a hybrid cell line from HUVEC and A549 (Edgell et al., 1983)) were cultured in DMEM supplemented with HAT. All media were supplemented with 10% FCS and 1% of each penicillin and streptomycin.

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