Recombinant adenovirus with enhanced green fluorescent protein

Ad-E3-EGFP

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Chemical name:	Recombinant adenovirus with enhanced green fluorescent protein	
Abbreviated name:	Ad-E3-EGFP	
Synonym:		
Agent Category:	Adenovirus	
Target:	Coxsackie-adenovirus receptor (CAR)	
Target Category:	Receptor	
Method of detection:	Fluorescence imaging, optical	
Source of signal:	Green fluorescence protein	
Activation:	Yes, viral replication required	
Studies:	• *In vitro* • Rodents	Click here for protein and nucleotide sequence of the Coxsackie-adenovirus receptor (CAR)

Background

[PubMed]

A variety of options, including surgery, radiation, chemotherapy, and biopharmaceuticals, or a combination of these therapies, have been developed and are commonly used for the treatment of cancer. However, these treatments often result in undesirable side effects for the patient and may not always be efficacious (1-3). As a result, the use of gene therapy as an alternative therapy for cancer has been developed and attempted by several investigators (4, 5). The adenovirus (Ad) is the most common vehicle used for the gene therapy of cancer, both as a vector and as an oncolytic agent (4, 5). The Ad is a non-

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enveloped DNA virus that binds to the Coxsackie-Ad receptor (CAR) on cells and has been studied for more than half a century. After binding to CAR, the virus interacts with the host cell integrins (which serve as secondary receptors) and is internalized by receptor-mediated endocytosis (6). A detailed description of Ad structure, gene composition, and biology is available elsewhere (7). Ad is one of the best-characterized viruses and, depending on the infecting serotype, is known to cause several different illnesses such as conjunctivitis, gastroenteritis, the common cold, and other respiratory ailments in humans (8, 9). There are ~50 serotypes of Ad, and among these Ad2 and Ad5 are the serotypes most commonly used for gene therapy. This is because Ad can be easily engineered to target a specific ailment such as cancer, can be made replication-deficient, does not integrate into the mammalian genome, can accommodate large transgenes, and can be modified to have a reduced immunological responses in mammals (10).

To make recombinant Ad suitable for use as a gene therapy vector, several strategies are used by investigators in the field (11). A common approach to generate an Ad vector is by substituting a viral gene(s) with the gene(s) of interest (11). For selective infection of cancerous tumors, conditionally replicating Ads (CRAds) have been generated that replicate only in the neoplastic cells and locally amplify the virus, which ultimately results in lysis of the host cell (thus the virus is oncolytic) (12). The lack of replication in normal tissue restricts the infection of CRAds to the cancerous cells, and the CRAds would replicate only until the cancerous cells are depleted. Other advantages of using CRAds for cancer therapy are discussed in detail by Kanerva and Hemminki (12). Although CRAds are being used in several clinical trials in the United States, still little is known regarding their replication, spreading ability, persistence, and interaction with the host system (13). According to Ono et al., this is probably because a non-invasive monitoring system was not available to investigate these CRAd parameters (13). Ono et al. envisioned that CRAds could probably be studied in an *in vivo* setting if the vector could be monitored with a fluorescent reporter gene (13).

This chapter details the development and *in vitro* and *in vivo* evaluation of a CRAd vector bearing the enhanced green fluorescent protein (EGFP) as the reporter gene (13).

Synthesis

[PubMed]

Synthesis of the recombinant CRAd described in this chapter was as detailed by Ono et al. (13). Using recombinant DNA and homologous recombination techniques, three vectors based on Ad5 were generated by deletion of the E3 region of the virus (for details of the Ad structure, see Rux and Burnett (7)). A commercially available plasmid was used as the source of the EGFP reporter gene inserted into each vector. Depending on the location of the EGFP gene in the recombinant virus genome, the vectors were designated as Ad-E3-EGFR F0, F1, and F2, respectively (13). The plasmids were then transfected into for initial vector production and subsequently propagated in A549 cells (14). After propagation and harvesting, the recombinant vectors were purified twice by ultracentrifugation on a

cesium chloride gradient and dialysis against phosphate-buffered saline containing magnesium, calcium, and glycerol. The titer of the viral particles and storage conditions for the final product were not reported by the investigators (13).

The EGFP signal could be detected with an excitation and emission bandpass filter combination with wavelengths of 490/10 and 535/30, respectively. The background autofluorescence was determined with a 560/10 and 605/55 combination filter. The background was subtracted from the fluorescence signal (13).

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

The *in vitro* expression of EGFP for the three recombinant constructs was monitored in A549 cells that support Ad5 replication (13). For this study, chemically transformed BALB/c mouse hepatoma cells (BNL-1NG-A.2) were used as controls because they are not conducive to Ad5 replication. Depending on the viral dose used to infect the cells, all the replication-competent viruses were observed to show an increase in fluorescence over time with the A549 cells. In the same conditions and time period, no increase in fluorescence was observed with the BNL-1NG-A.2 cells. An increase in fluorescence from the transfected cells was observed only if the virus was in a replication-permissive environment. At higher multiplicities of infection (1 and 10 virus particles/cell), there was a sigmoid increase in fluorescence. The investigators suggested that the sigmoid curve was observed because, after the initial infection, there was a log phase of infection of the surrounding cells that was followed by exhaustion and death (oncolysis) of the replication-permissive cells. Among the various constructs, the F2 construct showed the highest EGFP expression as determined by fluorescence monitoring.

In the same study, the increase in fluorescence in the A549 cells over 5 days was shown to be dose-dependent and correlated with replication of the F2 viral DNA (13). During the same period, no change in fluorescence was observed with the BNL-1NG-A.2 cells. From these observations the investigators concluded that the EGFP reporter system could be used for *in vitro* monitoring of Ad replication.

Animal Studies

Rodents

[PubMed]

A549 and BNL-1NG-A.2 cell tumors were established in athymic nude mice to evaluate the *in vivo* detection of Ad replication by fluorescence (13). A single injection of the F2 vector was administered directly into the tumors, and EGFP expression was detected and monitored with a non-invasive fluorescence optical imaging system. After imaging, the tumors were excised and the total Ad DNA copy number after replication was determined. A good correlation between the fluorescence and the DNA copy number was

reported for the A549 cells. No fluorescence was observed with the BNL-1NG-A.2 tumors. Replication of the F2 vector in the A549 tumors was monitored over 19 days. During the initial week, an increase in fluorescence from the tumor was observed, followed by a gradual decline in the signal, indicating viral clearance. The investigators reported that the area of infected cells did not spread beyond the initial region of the fluorescence signal and suggested that this was probably because of the inability of the virus to cause oncolysis of the entire tumor (13). The investigators cautioned that, although the EGFP system could be used for non-invasive, *in vivo* monitoring of Ad replication, its reduced detectability in deep tissue could be a limitation for clinical use.

Other Non-Primate Mammals

[PubMed]

No references are currently available.

Non-Human Primates

[PubMed]

No references are currently available.

Human Studies

[PubMed]

No references are currently available.

Supplemental Information

[Disclaimers]

No supplemental information is currently available.

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