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Members of adenovirus species B utilize CD80 and CD86 as cellular attachment receptors

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Abstract

Alternate serotypes of adenovirus (Ad), including Ads of species B, are being explored to circumvent the disadvantages of Ad serotype 5 gene delivery vectors. Whereas the majority of human Ads utilize the Coxsackievirus and adenovirus receptor (CAR), none of the Ad species B use CAR. Ad species B is further divided into two subspecies, B1 and B2, and utilizes at least two classes of receptors: common Ad species B receptors and B2 specific receptors. CD46 has been implicated as a B2-specific receptor. Ad serotype 3 (Ad3), a member of B1, utilizes CD80 and CD86 as cellular attachment receptors. The receptor-interacting Ad fiber-knob domain is highly homologous among species B Ads. We hypothesized that other members of Ad species B may utilize CD80 and CD86 as cellular attachment receptors. All tested species B members showed specific binding to cells expressing CD80 and CD86, and the Ad fiber-knob domain from both B1 and B2 Ad efficiently blocked CD80- and CD86-mediated infection of Ad3 vectors. Members of both B1 and B2 demonstrated CD80- and CD86-specific infection of CHO cells expressing CD80 and CD86. Therefore, all of the members of Ad species B utilize CD80 and CD86 for infection of cells.

Keywords: Adenovirus species B; Receptor; CD80; CD86

1. Introduction

Adenoviruses are non-enveloped, double-stranded DNA viruses with icosahedral symmetry. To date, there are 51 different human Ad serotypes, which are classified into six different species (A–F) based on their biological and genetic properties (Russell, 2000). Most Ad serotypes infect cells via a two-step process: (i) Ad binding to a primary cellular attachment receptor mediated by the distal knob domain of fiber protein extending from the Ad capsid vertices (Louis et al., 1994) and by (ii) the ensuing interaction of α_V integrins on the cell surface and the Ad penton base Arg-Gly-Asp (RGD) motif (Wickham et al., 1993). The initial binding of Ad to their primary receptor is thought to be one of the key determinants of virus tropism. Most human Ads cause disease in the respiratory tract, kidneys, eyes, intestine, or lymphoid tissue. The vast majority of the serotypes, including Ad serotypes 2 (Ad2) and 5 (Ad5), utilize CAR as their primary cellular attachment receptor (Bergelson et al., 1997; Roelvink et al., 1998; Tomko et al., 2000). Ad2 and Ad5 are the most thoroughly studied serotypes and the most commonly used Ad serotypes in gene therapy applications.

It is noteworthy that Ads have been exploited as gene transfer vectors. It is unfortunate that gene therapy protocols employing Ad5-based vectors have resulted in limited efficacies (Rancourt et al., 1998; Sterman et al., 1998). This may be in part due to deficient/low CAR expression or inaccessibility of CAR on the cell surface in the tissues of interest. CAR has been identified as a tumor suppressor protein (Kim et al., 2003; Okegawa et al., 2000) and a member of the intracellular junction (Cohen et

Abbreviations: Ad, adenovirus; Ad#, adenovirus serotype #; CAR, coxsackie and adenovirus receptor; CHO, Chinese hamster ovary; 6-HIS, six consecutive histidine residues; PBSCM, PBS with Ca and Mg; RGD, Arg-Gly-Asp; TBST, Tris buffered saline with Tween-20; sCD80, sCD86, Recombinant extracellular domains of human CD80 or CD86 fused with Fc region of human IgG1

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al., 2001; Walters et al., 2002). CAR is often down-regulated in neoplastic conversion; when expressed, its location basal to the tight junction may limit its accessibility. Efforts to circumvent these deficiencies have prompted the investigation of other non-CAR-binding Ad serotypes. One method of accomplishing such an investigation is by the construction of pseudotyped Ad vectors (Krasnykh et al., 1996). Pseudotyped Ad vectors replace the fiber-knob domain of the Ad5 vector with the fiber-knob domain from a non-CAR binding serotype, thereby achieving serotype-specific infection of cells. Vectors pseudotyped with the fiber-knob domain from serotypes of Ad species B, such as Ad serotype 3 (Ad3), efficiently transduce a variety of malignant cell types (Davidoff et al., 1999; Kanerva et al., 2002; Von Seggern et al., 2000) as well as many other target cells of interest (Stevenson et al., 1997). Until recently, further development and characterization of Ad species B-based vectors had been hindered by the failure to identify their primary cellular attachment receptors.

Species B is subdivided into two subspecies: B1, consisting of serotypes 3, 7, 16, 21, and 50; and B2, consisting of serotypes 11, 14, 34, and 35. There are at least two classes of species B Ad receptors: common species B Ad receptors utilized by all species B Ad and B2-specific receptors (Segerman et al., 2003a); however, none of the species B Ads utilize CAR as their primary cellular attachment receptor (Roelvink et al., 1998). Viruses from subspecies B2 can fully inhibit the binding of subspecies B1 viruses; however, viruses from subspecies B1 can only partially inhibit the binding of subspecies B2 viruses (Segerman et al., 2003a). CD46 was identified as a cellular attachment receptor for Ad serotypes 11 (Ad11) and 35 (Ad35), and both are members of the subspecies B2 (Gaggar et al., 2003; Segerman et al., 2003b). Additionally, all of subspecies B2 Ad as well as most of the subspecies B1 Ad are capable of binding to CD46 expressed on the cell surface. However, only binding of the virions to CD46 was demonstrated; transduction was not (Gaggar et al., 2003). Ad serotype 7 (Ad7), a member of subspecies B1, is capable of binding to CD46 but it cannot infect—CHO cells expressing CD46, leading to the hypothesis that CD46 serves as a subspecies B2-specific receptor (Segerman et al., 2003b). However, contrary to this hypothesis, Ad3 has recently been shown capable of using CD46 as a cellular attachment receptor, thereby complicating the picture of Ad species B receptors (Sirena et al., 2004). Furthermore, we have recently demonstrated that Ad3 is also capable of utilizing CD80 (B7.1) and CD86 (B7.2) as cellular attachment receptors (Short et al., 2004).

CD80 and CD86 are cell surface markers expressed on human dendritic cells and mature B-lymphocytes (Caux et al., 1994; Engel et al., 1994; Freeman et al., 1989; Lanier et al., 1995), and play a critical role in stimulating T-lymphocyte responses by their interaction with the ligands CD28 and CTLA-4 (Azuma et al., 1993; Caux et al., 1994; Lanier et al., 1995; Vasu et al., 2003). Both CD80 and CD86 are up-regulated during the process of dendritic cell maturation and are over-expressed in a variety of lymphocytic neoplasms and other neoplastic contexts (Koyama et al., 1998; Maeda et al., 2000; Mutti et al., 1998). Whereas CD80 and CD86 serve as a primary means of cellular entry for Ad3 on human dendritic cells, there may be additional cellular receptors for Ad3 capable of mediating the infection of HeLa cells (Short et al., 2004).

To better understand the cellular receptors utilized by Ad species B, we investigated other serotypes of Ad species B for their ability to utilize CD80 and CD86. The Ad fiber-knob domain is highly homologous among species B Ad. On this basis, we hypothesized that other members of Ad species B may utilize CD80 and CD86 as cellular attachment receptors.

2. Materials and methods

2.1. Cell culture

Chinese hamster ovary (CHO) cell line and HeLa cell line (both from ATCC, Manassas, VA) were grown in DMEM/F12 50:50 with 10% FBS and antibiotics as recommended by the provider. Generation of CHO-CD80 and CHO-CD86 cell lines and characterization of ligand expression levels were reported earlier (Short et al., 2004; Vasu et al., 2003). The cells were grown in the same media in the presence of geneticin at 400 μg/ml. All cells were cultured in a humidified incubator at 37 °C with 5% CO_2.

2.2. Wild-type and recombinant Ad

Wild-type Ad serotypes 3, 5, 7, 11, 14, 16, 21, 34, 35, and 50 (ATCC) were amplified and titrated spectrophotometrically (Maizel et al., 1968). Two replication-incompetent Ad5-based vectors containing a firefly luciferase transgene cassette in place of the deleted E1 region were used. Ad5luc1 contains the full Ad5 capsid and fiber proteins whereas Ad5/3luc1 contains the Ad5 capsid with chimeric fiber proteins in which the tail and shaft domains are from Ad5 and the knob domain is from Ad3. Both vectors were generated in our laboratory and described previously (Kanerva et al., 2002).

2.3. Recombinant Ad3 and Ad5 fiber-knob domain and Ad7 and Ad11 fiber protein production

The knob domains of Ad5 and Ad3 fibers (Krasnykh et al., 1996) and the fibers of Ad7 and Ad11 were produced in *E. coli* with N-terminal tags of six consecutive histidine residues (6-HIS), using the pQE30 expression vector (Qiagen, Valencia, CA). Ad7 and Ad11 wild-type genomes were used as templates for PCR to amplify the fiber genes of the respective serotype. Primers for these reactions were Ad7F11F.F (5′-TAC CCC GGG AAT GAC CAA GAG AGT CCG G-3′), Ad7F.R (5′-ATT AAG CTT TCA GTC GTC TTC TCT AAT G-3′), Ad11F.R (5′-ATT AAG CTT TCA GTC GTC TTC TCT GAT G-3′). The same forward primer was used in both reactions but with each reverse primer a DNA sequence was amplified coding for the fiber polypeptide of the respective serotype. Both PCR products were then digested with *Sma* I and *Hind* III and cloned into *Sma* I-*Hind* III-digested pQE30, resulting in plasmids pQE.Ad7F and pQE.Ad11F further used to transform *E. coli*. The soluble forms of the Ad3 and Ad5 fiber-knob domain and Ad7 and Ad11 fiber proteins were isolated from the induced