Virology 502 (2017) 144–151

Contents lists available at ScienceDirect

Virology

journal homepage: www.elsevier.com/locate/yviro

Adenovirus transduction: More complicated than receptor expression

Priyanka Sharma[a], Prithy C. Martis[b], Katherine J.D.A. Excoffon[a,b,*]

[a] Department of Biological Sciences, Wright State University, Dayton, OH, USA
[b] Biomedical Sciences PhD Program, Wright State University, Dayton, OH 45435, USA

ARTICLE INFO

Keywords:
Adenovirus
Coxsackievirus and adenovirus receptor (CAR)
Non-polarized
Polarized
Tight junction
Epithelia
Apical and basolateral

ABSTRACT

The abundance and accessibility of a primary virus receptor are critical factors that impact the susceptibility of a host cell to virus infection. The Coxsackievirus and adenovirus receptor (CAR) has two transmembrane isoforms that occur due to alternative splicing and differ in localization and function in polarized epithelia. To determine the relevance of isoform-specific expression across cell types, the abundance and localization of both isoforms were determined in ten common cell lines, and correlated with susceptibility to adenovirus transduction relative to polarized primary human airway epithelia. Data show that the gene and protein expression for each isoform of CAR varies significantly between cell lines and polarization, as indicated by high transepithelial resistance, is inversely related to adenovirus transduction. In summary, the variability of polarity and isoform-specific expression among model cells are critical parameters that must be considered when evaluating the clinical relevance of potential adenovirus-mediated gene therapy and anti-adenovirus strategies.

1. Introduction

Understanding the development, maintenance, and composition of the lung epithelial barrier is of great importance to human respiratory health and disease. This barrier segregates the microbial infested external environment from the body's sterile internal environment. Each polarized epithelial cell has two distinct compartments: an apical or mucosal surface exposed to the air, and a basolateral surface in close communication with the internal environment. Investigations into the mechanisms of pathogenic microbial penetration of the lung epithelial barrier provide insights into its structure and regulation (Zihni et al., 2014). Interestingly, many viruses use basolateral adhesion proteins as their primary receptors. Three groups of viruses, coxsackie B viruses (CVB), all but group B human adenoviruses, and swine vesicular disease virus, utilize the coxsackievirus and adenovirus receptor (CAR) as their primary receptor (Bergelson et al., 1997; Carson et al., 1997; Tomko et al., 1997).

Several protein isoforms have been described for CAR (Excoffon et al., 2014, 2010; Raschperger et al., 2006). Alternative splicing not only regulates protein expression, but also allows multiple proteins to be expressed from the same gene resulting in significant proteomic diversity. Human CAR was initially isolated as a seven exon encoded protein (Bergelson et al., 1997). In contrast to other species, mouse CAR (mCAR) was initially cloned as a protein composed of eight exons (Tomko et al., 1997). Transcripts for the seven-exon mouse and eight-exon human forms were subsequently identified (Bergelson et al., 1998). A detailed analysis of protein expression and localization in mice has revealed differential tissue dependent expression and localization for the $mCAR^{Ex7}$ (mCAR2) and $mCAR^{Ex8}$ (mCAR1) isoforms (Excoffon et al., 2010; Mirza et al., 2006; Nalbantoglu et al., 2001, 1999; Raschperger et al., 2006). This suggests that protein-protein interactions and potentially the functional importance of these two isoforms may be distinct. We are the first group to investigate the importance of human CAR^{Ex8} in polarized epithelia (Excoffon et al., 2010; Kolawole et al., 2012; Kotha et al., 2015; Sharma et al., 2012a, b; Yan et al., 2015). The alternative splicing event that creates CAR^{Ex8} occurs at a cryptic splice site within the seventh exon. Thus, these two isoforms contain identical extracellular and transmembrane domains. The majority of the cytoplasmic C-terminal domain is also identical except for the last 26 amino acids encoded by exon 7 (CAR^{Ex7}) that are replaced by 13 distinct amino acids encoded by exon 8 (CAR^{Ex8}).

Along with others, we have previously shown that CAR^{Ex7} resides on the basolateral surface of polarized epithelia where it behaves as a homophilic epithelial junction adhesion protein and a heterophilic leukocyte adhesion protein that plays a role in neutrophil transepithelial migration and gammadelta T cell activation (Cohen et al., 2001; Excoffon et al., 2010; Kotha et al., 2015; Verdino et al., 2010; Walters et al., 2002; Witherden et al., 2010; Zen et al., 2005). CAR^{Ex7} also plays a role in trafficking proteins to cell-cell junctions (Coyne et al., 2004; Excoffon et al., 2004, 2012; Lim et al., 2008; Mirza et al., 2005; Sollerbrant et al., 2003; Weber et al., 2014; Yan et al., 2015).

CAR^{Ex8} can also behave as a homo- and heterophilic adhesion

* Corresponding author at: Department of Biological Sciences Wright State University, 3640 Colonel Glenn Hwy, Dayton, OH 45435, USA.
E-mail address: katherine.excoffon@wright.edu (K.J.D.A. Excoffon).

http://dx.doi.org/10.1016/j.virol.2016.12.020
Received 30 September 2016; Received in revised form 2 December 2016; Accepted 19 December 2016
Available online 31 December 2016
0042-6822/

protein; however, in contrast to CAR^{Ex7}, CAR^{Ex8} is found at the apical surface of polarized epithelia where it is unlikely to interact with CAR molecules on adjacent cells (Excoffon et al., 2010). Instead, we have shown that CAR^{Ex8} mediates heterophilic adhesion with neutrophils that have transmigrated from the basal to apical surface (Kotha et al., 2015). The apical CAR^{Ex8} isoform also mediates adenovirus entry from the apical surface and, surprisingly, neutrophils further enhance adenovirus entry. The protein levels of apical CAR^{Ex8} are tightly regulated by intracellular proteins, such as the cytoplasmic scaffolding protein MAGI-1, and are increased by extracellular signals such as interleukin-8 (IL-8) and side-stream cigarette smoke (Excoffon et al., 2010; Kolawole et al., 2012; Kotha et al., 2015; Sharma et al., 2012a).

The abundance and accessibility of a primary viral receptor is a critical factor that defines the susceptibility of a host cell to viral infection. Many distinct model cells have been investigated for CAR-binding virus infection. In this manuscript we characterize cellular polarity and isoform-specific expression of both transmembrane isoforms of CAR in eleven commonly studied cell types. The cell lines tested were divided in two groups based on their polarity; 1) non-polarized cells (CHO-K1, COS-7, HeLa, HEK-293, 293 T and A549) that are unable to form tight junctions and hence unable to polarize at the air-liquid interface; 2) cells able to form tight junctions (MDCK, Caco-2, Calu-3, NuLi-1 and HAE) and therefore polarize on a semipermeable membrane resulting in two distinct compartments (apical and basolateral). Together, these data will allow those investigating CAR-binding virus infections a clearer understanding of cellular polarity and receptor availability that will enhance data interpretation and advance the understanding of the factors that regulate susceptibility to infection.

2. Materials and methods

2.1. Cell lines

Ten immortal cell lines and primary human airway epithelia were investigated: Chinese hamster ovary (CHO-K1, ATCC, CCL-61), transformed monkey kidney (COS-7, ATCC, CRL-1651), human cervical adenocarcinoma (HeLa, ATCC- CCL-2), human embryonic kidney (HEK-293, ATCC, CRL-1573), subclone of human embryonic kidney (293 T, Clontech, Mountain View, CA), human lung carcinoma (A549, ATCC, CCL-185), Madin Darby canine kidney (MDCK, ATCC, CCL-34), human colonic adenocarcinoma (Caco-2, ATCC, HTB-37), human lung adenocarcinoma (Calu-3, ATCC, HTB-55), normal lung transformed epithelial cells (NuLi-1, a kind gift from Dr. Joseph Zabner, University of Iowa) and human airway epithelial (HAE, Human Donors).

HAE were originally isolated under approval by the Institutional Review Board of the University of Iowa (IRB ID No. 9507432) from discarded and deidentified trachea and bronchi of donor lungs. This study used discarded lung tissue, thus IRB deemed consent was not needed. HAE were cultured and differentiated as previously described (Karp et al., 2002; Kotha et al., 2015; Liu et al., 2012).

2.2. Culture media

The cells were cultured in appropriate sterile culture medium (Gibco™, Invitrogen Corporation, Grand Island, NY) supplemented with 10% fetal bovine serum (except MDCK, supplemented with 5% FBS). All the culture media were further supplemented with 0.5% penicillin and streptomycin antibiotics and with 3.024 g/L sodium bicarbonate.

2.3. Total cellular RNA extraction

Total cellular RNA was extracted from cells using TRIzol Reagent (Invitrogen Life Technologies, Carlsbad, CA) according to the manufacturer's protocol. The RNA pellet was dissolved in diethyl pyrocarbonate (DEPC)-treated ddH_2O and the purity and concentration of the extracted RNA were determined with a nanodrop spectrophotometer (Thermo Scientific, Waltham, MA).

2.4. Quantitative reverse transcription and PCR (qPCR)

Complementary DNA (cDNA) was synthesized using Quanta First Strand Kit (Quanta BioSciences, Gaithersburg, MD). qPCR was performed using SYBR Green with low ROX (Quanta BioSciences) in Stratagene's Real Time PCR System (Agilent Technologies, Santa Clara, CA) using 3000p software v2.0.1 for data analysis as previously described (Kotha et al., 2015; Sharma et al., 2012a, 2012b). Primers used were: CAR-qPCR-F: 5′ TCGGCAGTAATCATTCATCCCTGG; CAR^{Ex7}-qPCR-R: 5′ ATAGACCCATCCTTGCTCTGTGCT;CAR^{EX8}-qPCR-R: 5′ ACTGTAATTCCATCAGTCTTGTAAGGG. Abundance relative to GAPDH gene expression was calculated for each gene of interest in human cells. GAPDH-F: 5′ CACCCTGTTGCTGTAGCCAAA; GAPDH-R: 5′ CAACAGCGACACCCACTCCT. In dog cells, CAR was normalized to MDCK-Actin-F: 5′ AAGATCTGGCACCACACCTTCTAC; MDCK-Actin-R: 5′ ATCTGGGTCATCTTCTCACGGTTG (Kotha et al., 2015). In CHO cells, CAR was normalized to CHO-Actin-F-5′ TGGCATCCACGAAACTACAT; CHO-Actin-R-5′ TGGTACCACCAGACAGCACT.

2.5. Immunocytochemistry

Cells seeded and polarized on millicells (0.4 μm pore; Millipore, St. Louis, MO) were kept on ice for 5 min, and washed 3 times with ice-cold PBS supplemented with Mg^{2+} and Ca^{2+}(PBS +/+). The cells were then fixed with ice-cold methanol containing 1% paraformaldehyde for 20 min at −20 °C. Cells were rinsed with ice-cold PBS +/+, allowed to come to room temperature, and blocked with 2% bovine serum albumin (BSA) in SuperBlock (Pierce, Rockford, IL) for 45 min. Primary antibodies, anti- rabbit 1605 (rα1605) for total CAR, anti-rabbit 5678 (rα5678) and anti-mouse actin as previously published (Excoffon et al., 2014, 2010, 2005) were added to cells for 2.5 h at 37 °C or overnight at 4 °C. Cells were rinsed, reblocked, and then incubated with secondary antibodies (Invitrogen, Carlsbad, CA). The cells were rinsed and mounted onto glass slides using Vectashield mounting media with DAPI (Vector Laboratories Inc., Burlingame, CA). Staining was evaluated by laser scanning confocal microscopy (Olympus FV1000) with a 60X oil immersion lens; images are shown as single X-Y sections.

2.6. Cell Polarization

For polarization studies, 2×10^4–2×10^6 cells per well were seeded on 12 mm diameter polyester Millicell filters consisting of a semi-permeable membrane with a pore size of 0.4 μm (Millipore, St. Louis, MO). Media on the apical surface of cells was removed every alternate day in order to establish and maintain an air-liquid interface. Polarized cells actively transport fluid from the apical to the basolateral surface and thus maintain a defined apical surface fluid composition.

2.7. Transepithelial electrical resistance (TER) measurement

Transepithelial electrical resistance was measured with a chopstick ohmmeter (World Precision Instruments, Sarasota, FL) every other day. Media was aspirated from the wells and replaced with 400 μL fresh media at the basolateral surface. The same amount of PBS +/+ was applied to the apical surface. The background electrical resistance was determined by adding media to the basolateral chamber and PBS +/+ to the apical chamber of a blank Millicell filter. The TER measurements were recorded as $\Omega\cdot cm^2$.