Journal of Controlled Release 237 (2016) 78–88

Contents lists available at ScienceDirect

Journal of Controlled Release

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

Albumin-binding adenoviruses circumvent pre-existing neutralizing antibodies upon systemic delivery

Luis Alfonso Rojas [a], Gabriela N. Condezo [b], Rafael Moreno Olié [a], Carlos Alberto Fajardo [a], Marcel Arias-Badia [a], Carmen San Martín [b], Ramon Alemany [a,*]

[a] Virotherapy and Gene therapy Group, ProCure Program, Instituto Catalan de Oncología-IDIBELL, L'Hospitalet de Llobregat 08908, Spain
[b] Department of Macromolecular Structure, Centro Nacional de Biotecnología (CNB-CSIC), Madrid 28049, Spain

ARTICLE INFO

Article history:
Received 21 March 2016
Received in revised form 30 June 2016
Accepted 2 July 2016
Available online 04 July 2016

Keywords:
Adenovirus
Albumin
Albumin-binding domain
Neutralizing antibodies

ABSTRACT

Recombinant adenoviruses are used as vaccines, gene therapy vectors, and oncolytic viruses. However, the efficacy of such therapies is limited by pre-existing neutralizing antibodies (NAbs), especially when the virus is administered systemically for a wider biodistribution or to reach multiple metastases. To protect adenovirus against NAbs we inserted an albumin-binding domain (ABD) in the main adenovirus capsid protein, the hexon. This domain binds serum albumin to shield the virus upon systemic administration. The ABD-modified adenoviruses bind human and mouse albumin and maintain the infectivity and replication capacity in presence of NAbs. In pre-immunized mice non-modified viruses are completely neutralized, whereas ABD-modified viruses preserve the ability to transduce target organs, induce oncolysis, or generate immune responses to expressed proteins. Our results indicate that albumin coating of the virus capsid represents an effective approach to evade pre-existing NAbs. This strategy has translational relevance in the use of adenovirus for gene therapy, cancer virotherapy, and vaccination.

1. Introduction

Oncolytic viruses have shown promising clinical results in the last few years. Several viruses are currently in phase II and III clinical trials [1], and the modified herpes simplex virus T-vec has recently been approved by the FDA and EMA for melanoma treatment by intratumoral administration. Nonetheless, in general terms pre-existing humoral immunity represents a major hurdle for efficacy, not only in cancer virotherapy but also in the fields of gene therapy vectors and vaccination [2,3], specially upon intravenous or systemic administration. The human adenovirus serotype 5 (Ad5) is the most commonly used for adenoviral virotherapy, however the prevalence of anti-Ad5 neutralizing antibodies (NAbs) is very high in the general population, reaching >90% in certain geographical locations [4,5]. Several studies have demonstrated that Ad5-specific NAbs are directed primarily against the hypervariable regions (HVRs) of hexon protein [6,7].

Multiple strategies have been explored to evade NAbs. Chemical conjugation of capsid with polymers such as polyethyleneglycol or N-(2-hydroxypropyl)methacrylamide shields the virus at the expense of virus infectivity [8,9]. Furthermore, a conjugated product increases the complexity of GMP production for clinical application. Use of non-prevalent human serotypes or simian serotypes usually underperform Ad5 vectors [10,11] and imply GMP production challenges as well. Replacement of Ad5 HVRs with those from non-prevalent serotypes has shown promising results in vaccination [12,13], but it has not been tested with oncolytic adenoviruses. In addition, Ad5 HVR1 recruits dynein for proper capsid transport to nucleus and when substituted by HVR1 of Ad48 capsid trafficking is affected [14].

The use of albumin as a drug carrier is a hot research field, recently leading to the approval of Abraxane® and Levemir® to treat cancer and diabetes, respectively [15]. Taking advantage of its long plasma half-life, non-covalent interaction with albumin has been shown to reduce the blood clearance of short-lived drugs [16–18]. Albumin acts also as a tumor-targeting carrier as it accumulates in solid tumors [15]. We aimed to use albumin binding as a protection mechanism against NAbs. To generate an albumin-binding adenovirus we have used the albumin-binding domain 3 (ABD) from the protein G of *Streptococcus* [19–21]. This domain was inserted in the HVR1 of adenovirus hexon, and flanked by two GSGS-linkers to confer flexibility. We generated both an oncolytic adenovirus (ICOVIR15-ABD) and an E1-deleted GFP-Luciferase vector (AdGLRGD-ABD) with ABD. We hypothesized that upon injection of the modified virus in the bloodstream albumin

* Corresponding author at: IDIBELL-Institut Català d'Oncologia, Av Gran Via de l'Hospitalet 199-203, L'Hospitalet de Llobregat 08907, Barcelona, Spain.
E-mail address: ralemany@iconcologia.net (R. Alemany).

http://dx.doi.org/10.1016/j.jconrel.2016.07.004
0168-3659/

would coat the viral capsid, increasing its plasma half-life and shielding it against circulating NAbs (Fig. 1A).

2. Results

2.1. ABD insertion promotes albumin binding

Parental oncolytic adenovirus ICOVIR15 has been described [22] and was used as a control. The albumin-binding domain 3 from the protein G of *Streptococcus* was inserted in the HVR1 of ICOVIR15 hexon, generating the oncolytic adenovirus ICOVIR15-ABD. The domain was flanked by GSGS-linkers and centered in HVR1 after the D150 amino acid without deleting any hexon sequence (Fig. 1B). The insertion was also introduced in the reporter non-replicative vector AdGLRGD, obtaining AdGLRGD-ABD.

To demonstrate the functionality of the ABD in adenovirus capsid, we tested the binding of ICOVIR15-ABD to purified human (HSA), mouse (MSA), and bovine serum albumin (BSA) by ELISA. Positive binding was observed when adding ICOVIR15-ABD to HSA- or MSA-coated wells (Fig. 2A). In agreement with the binding pattern of ABD to albumin of different species [23,24], the virus did not bind to BSA. As expected, the parental virus ICOVIR15 did not bind to any albumin. The ABD-modified virus was also able to bind to albumin in human and mouse serum in a dose-dependent manner (Fig. 2B), contrary to the non-modified virus which did not show any binding. Binding of ICOVIR15-ABD to HSA was confirmed by immunoelectron microscopy since positive albumin staining was only observed in ICOVIR15-ABD capsids (Fig. 2C). Cryo-electron microscopy studies showed additional electron density in the vicinity of the HVR1 of ICOVIR15-ABD, compatible with the presence of bound albumin (Fig. 2D–E).

To understand how the ABD insertion affected the viability of the virus we studied the virus life cycle in absence of NAbs. We observed a minor loss of ICOVIR15-ABD production yields (2–3 times) compared to the non-modified ICOVIR15 (Fig. 3A) in A549 cells (e.g. 4900 TU/cell ICOVIR15 vs 1500 TU/cell ICOVIR15-ABD at 72 h post-infection, $p = 0.00083$). Infectivity and cytotoxicity were analyzed in presence and absence of HSA. No differences in infectivity were observed among vectors in a panel of tumor cell lines in the absence of albumin. However, albumin caused a 2-fold loss of transduction in the non-modified vector in most of the cell lines, and an even a higher impairment for the ABD-vector (20-fold in A549, 10-fold in NP-9 and Sk-mel28, 6-fold in B16, and 3-fold in B16-CAR, Fig. 3B). No differences in cytotoxicity were observed among viruses in absence of HSA. With HSA in the medium, parental virus ICOVIR15 suffered a 2-fold loss of cytotoxicity, compared to a 6-fold loss for ICOVIR15-ABD (Fig. 3C). In general terms, these results indicate an attenuation of the ABD-modified virus in presence of albumin.

2.2. Albumin binding protects adenovirus from neutralizing antibodies in vitro

Having demonstrated the binding of ICOVIR15-ABD to HSA, we tested if this binding could protect the virus from NAbs in vitro. AdGLRGD or AdGLRGD-ABD in absence or presence of HSA, were incubated with serial dilutions of the commercial neutralizing antibody Ab6982 (rabbit polyclonal against Ad5), and then used to transduce A549 cells. Even in absence of human albumin the modified AdGLRGD-ABD was less neutralized than the non-modified vector (Fig. 4A), suggesting that the ABD modification of the HVR1 precluded binding of some NAbs, likely directed against the wt HVR1. In presence of albumin, the transduction efficiency of AdGLRGD-ABD was remarkably enhanced (e.g. 8% of transduction of AdGLRGD-ABD vs 70% of transduction of AdGLRGD-ABD + HSA at 1/64 dilution of Ab6982, $p = 0.000011$), indicating that albumin was able to protect the virus from NAbs. Addition of HSA did not alter the transduction of the non-modified vector AdGLRGD, which was efficiently neutralized.

In addition, the capacity of viruses to kill cancer cells in presence of NAbs was also evaluated. For this purpose, ICOVIR15 or ICOVIR15-ABD in absence or presence of HSA were incubated with serial dilutions of Ab6982, and used to infect A549 cells. Cell survival was analyzed 5 days after infection. In absence of HSA both viruses showed similar capacity to kill tumor cells (Fig. 4B), and only a small increase of cytotoxicity was observed with ICOVIR15-ABD probably due to the certain evasion of NAbs observed in transduction (Fig. 4A). Importantly, HSA shifted the cytotoxicity curve of ICOVIR15-ABD towards higher NAbs concentrations (e.g. 75% of cell survival of ICOVIR15-ABD vs 8% of cell survival of ICOVIR15-ABD + HSA at 1/32 dilution of Ab6982, $p = 0.000015$), whereas it did not protect ICOVIR15 from neutralization.

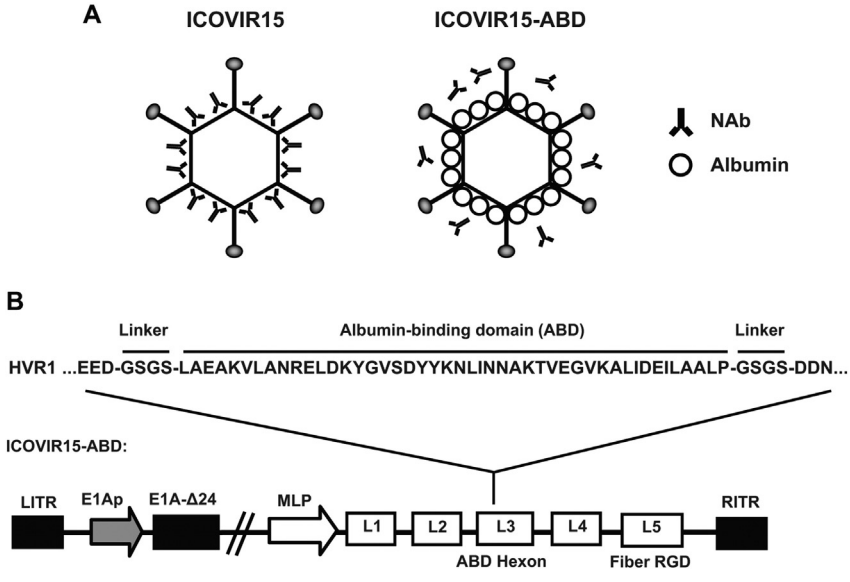

Fig. 1. Design of an oncolytic adenovirus containing an albumin-binding domain (ABD) inserted in the hexon. A, Albumin-protection conferred by ABD insertion. Compared to a non-modified adenovirus ICOVIR15 (left), the ABD-modified virus ICOVIR15-ABD (right) is coated with albumin present in blood, shielding the virus from neutralizing antibodies (NAbs). B, schematic diagram of ABD insertion in ICOVIR15-ABD genome. The ABD 3 from streptococcal protein G is flanked by two GSGS linkers and inserted in the middle of the HVR1 of hexon of ICOVIR15 obtaining ICOVIR15-ABD. LITR/RITR, left and right inverted terminal repeats; MLP, major late promoter; E1Ap, modified E1A promoter; E1A-Δ24, mutant version of E1A protein with deletion of aminoacids 121–129; L1 to L5, late genes; Fiber RGD, modified fiber by insertion of the RGD peptide at the HI-loop.