Identification of CD46 Binding Sites within the Adenovirus Serotype 35 Fiber Knob

Imameddin Amiraslanov and Yen-Chywan Liaw (廖彥銓)

Institute of Molecular Biology, Academia Sinica, Taipei, Taiwan

Human adenoviruses (Ads) have been classified into six species (A to F), containing 51 serotypes. Species B Ads form two genetic clusters, B1 (Ad serotype 3 [Ad3], Ad7, Ad16, Ad21, and Ad50) and B2 (Ad11, Ad14, Ad34, and Ad35). Most B1 serotypes are associated with acute respiratory disease and, unlike the species C Ads (e.g., Ad5), do not establish persistence. The B2 serotypes are mainly associated with infections of the kidney and urinary tract and are often fatal in immunocompromised individuals. Species B Ads can also be grouped based on their receptor usage. Group I Ads (Ad serotypes 16, 21, 35, and 50) nearly exclusively use CD46, a ubiquitously expressed membrane protein with complement regulatory functions that is upregulated on tumor and stem cells. Group II Ads (Ad serotypes 3, 7, and 14) utilize a still unidentified receptor X, but not CD46. Group III (Ad11) uses both CD46 and receptor X. Recently, gene transfer vectors based on species B Ads have shown promise for cell and gene therapy. Vectors derived from species B Ads or Ad5 vectors containing fibers from species B Ads efficiently transduce human cell types that are relatively refractory to infection with classical serotype Ad5 vectors, including tumor cells, hematopoietic stem cells, mesenchymal stem cells, dendritic cells, and lymphocytes. The most commonly used species B vectors contain Ad35 fibers (Ad35 or Ad5/F35), and therefore most studies on the interaction of species B Ads with CD46 have focused on Ad35. Ad35 binds through its trimeric fiber knob to CD46 with a high avidity. While the interacting residues within CD46 have been localized to the two distal extracellular domains of CD46 (SCR1 and SCR2), the contact areas within the Ad35 knob have not been reported so far.

The structure of an Ad fiber knob domain was first determined in 1994, for the species C serotype Ad5, which uses the coxsackie and adenovirus receptor (CAR) for attachment. The homotrimeric knob structure is best described as a three-bladed propeller, whereby each blade contains multiple tightly packed beta-sheets (labeled A to J). The structure of a CD46-interacting Ad knob (Ad11), which is similar overall to other Ad knobs, was recently published. Crystallization of recombinant Ad11 knob bound to CD46 domains SCR1 and SCR2 revealed three critical contact regions within the FG, HI, and IJ loops of the fiber knob. This model is supported by studies demonstrating that binding of Ad11 virus to CD46 can be abolished by introduction of a single amino acid substitution (Arg279Gln) within the Ad11 HI loop. Although there is overlap in tropism between Ad11 and Ad35, Ad11 virus binds to CD46 with a higher avidity, which implies that the mechanism of Ad11-CD46 interaction cannot necessarily be translated to Ad35. In this study we show the affinity of the Ad35 knob to CD46, report the crystal structure for the Ad35 knob, identify four amino acids in the Ad35 knob that are critical for CD46 binding, and present a model for Ad35 knob interaction with CD46.

To study the Ad35 knob-CD46 interaction, we generated an expression library of Ad35 knobs with random mutations and screened it for CD46 binding. We identified four critical residues (Phe242, Arg279, Ser282, and Glu302) which, when mutated, ablated Ad35 knob binding to CD46 without affecting knob trimerization. The functional importance of the identified residues was validated in surface plasmon resonance and competition binding studies. To model the Ad35 knob-CD46 interaction, we resolved the Ad35 knob structure at 2-Å resolution by X-ray crystallography and overlaid it onto the existing structure for Ad11-CD46 interaction. According to our model, all identified Ad35 residues are in regions that interact with CD46, whereby one CD46 molecule binds between two knob monomers. This mode of interaction might have potential consequences for CD46 signaling and intracellular trafficking of Ad35. Our findings are also fundamental for better characterization of species B Ads and design of antiviral drugs, as well as for application of species B Ads as in vivo and in vitro gene transfer vectors.