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Adenovirus-host interactions: implications for tropism and therapy

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Akademisk avhandling

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Abstract
Human adenoviruses (HAdVs) are common viruses often associated with gastrointestinal, ocular and respiratory infections. They can infect a wide variety of cells, both dividing and non-dividing. HAdVs attach to and infect target cells through interactions with cellular receptors. They can also utilize soluble host components in body fluids for indirect binding to target cells, a feature that enables the usage of new types of receptors resulting in a more efficient HAdV infection. Here we demonstrate that HAdV-5 (species C) infection is enhanced by plasma, saliva and tear fluid and HAdV-31 (species A) infection is enhanced by plasma. We further pinpoint the increase in infection seen with plasma to be mediated by coagulation factor IX (FIX) and X (FX) for HAdV-5 and FIX for HAdV-31. We found that as little as 1% of the physiological concentration of these factors was enough to reach maximum increase in binding.

This study was then extended to include all HAdVs in species A: HAdV-12, 18 and -31. Species A normally cause mild respiratory or gastrointestinal infections, but in recent decades species A in general, and HAdV-31 in particular, have been shown to cause life-threatening infections in immunocompromised patients. We could show that FIX mediated increase of both HAdV-18 and -31 infections, while no effects was seen for HAdV-12. FIX was further shown to interact with the hexon protein of HAdV-31 and surface plasmon resonance analysis revealed that the HAdV-31:FIX interaction was slightly stronger than that of the HAdV-5:FIX/FX interactions, but more interestingly, the half-lives of these interactions were profoundly different, a feature that we believe could be one explanation for the difference in binding to cellular heparan sulfate seen with these complexes.

We conclude that the use of coagulation factors could be of importance not only for the liver accumulation seen when administering HAdV-5 based vectors into the circulation, but also for the natural tropism of these viruses. We also believe that our findings may contribute to better design of HAdV-based vectors for gene and cancer therapy and that the interaction between HAdV-31 hexon and FIX may serve as a target for antiviral treatment.

HAdV-52 is one of only three HAdVs that are equipped with two different fiber proteins, one long and one short. We show here, by means of binding and infection experiments, that HAdV-52 can use CAR as a cellular receptor, but that most of the binding is dependent on sialic acid (Sia)-containing glycoproteins. Flow cytometry, ELISA and surface plasmon resonance analyses revealed that the terminal knob domain of the long fiber (52LFK) binds to CAR, and the knob domain of the short fiber (52SFK) binds to sialylated glycoproteins. X-ray crystallographic analysis of 52SFK in complex with Sia revealed a new Sia-binding site compared to other known adenovirus:glycan interactions.

This study was then extended to characterize the Sia-containing glycan used by 52SFK in more detail. Glycan array screening showed that 52SFK bound specifically to long chains of α2,8-linked (poly) sialic acids (PSia). Cell based binding experiments confirmed a strong preference for 52SFK binding to cells displaying high levels of PSia compared to control cells. X-ray crystallograpic analysis of 52SFK in complex with oligo-PSia revealed engagement at the non-reducing end of oligo-Sia to the canonical Sia-binding site on the fiber, but also suggested the presence of a 'steering rim' consisting of positively charged amino acids contributing to the interaction. From this we conclude that HAdV-52 can use both CAR and Sia-containing glycans as cellular receptors. 52SFK binds specifically to PSia, a glycan close to absent on cells in healthy adults but re-expressed on several types of cancers. We therefore believe that HAdV-52-based vectors could be useful for treatment of cancer types with elevated PSia-expression.

Keywords
Adenovirus, receptor, coagulation factor, sialic acid, therapy

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