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NOTES

Influence of Coagulation Factor Zymogens on the Infectivity of Adenoviruses Pseudotyped with Fibers from Subgroup D[∇]

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Recent evidence supports a role for vitamin K-dependent coagulation zymogens in adenovirus serotype 5 (Ad5, subgroup C) infection of hepatocytes. Here, we assessed the effect of virus-zymogen interaction on cellular transduction using a panel of fiber (f)-pseudotyped viruses derived from subgroup D (f47, f33, f24, f45, f17, f30). Each virus directly bound factor X (FX) as determined by surface plasmon resonance, resulting in enhanced cell surface binding. Infection of HepG2 cells was promoted by FX but not by FVII or FIX, while transduction of CHO cells was blocked in heparan sulfate proteoglycan-deficient cells. This suggests a broad role for FX in adenovirus infectivity.

Adenoviral vectors, particularly adenovirus serotype 5 (Ad5, subgroup C virus), are used routinely for gene therapy in the experimental setting and are being tested extensively in the clinical setting. In vitro, Ad5 binds to cells predominantly

through primary interaction with the coxsackie virus and adenovirus receptor (3, 18) and internalization through integrin engagement (19). The profound in vivo infectivity of Ad5 for the liver following systemic delivery, however, is dictated by the

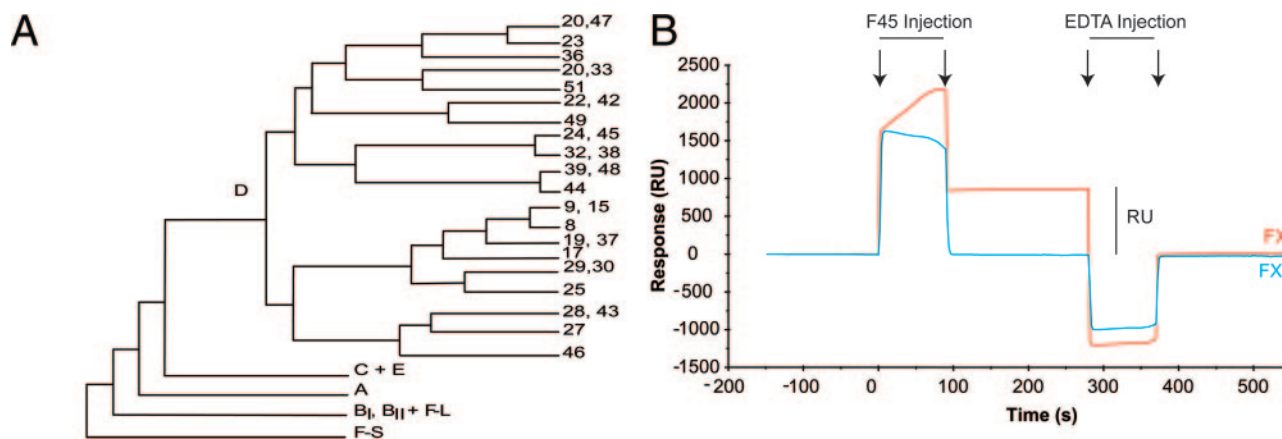


FIG. 1. Subgroup D viruses and surface plasmon resonance analysis of FX-virus binding. (A) Phylogenetic depiction of subgroup D viruses, adapted from previous work (9). (B) Adenovirus Ad5/f45 was perfused over FX, and FXI was immobilized onto a CM5 sensor chip in 50 mM Tris (pH 7.4), 150 mM NaCl, 5 mM CaCl₂, 0.005% Tween 20 at a flow rate of 20 μ l/m at 25°C. Shown are typical sensorgrams indicating an association with FX but not FXI and an undetectable dissociation of the virus from FX following the end of the injection but a ready dissociation upon injection of 3 mM EDTA. RU, response units.

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virus binding to coagulation zymogens, including factor IX (FIX) (16) and FX (15). This is particularly relevant for understanding adenovirus infectivity following intravascular administration, since the virus comes into immediate contact with blood cells (14) and circulating proteins. For efficient retarget-

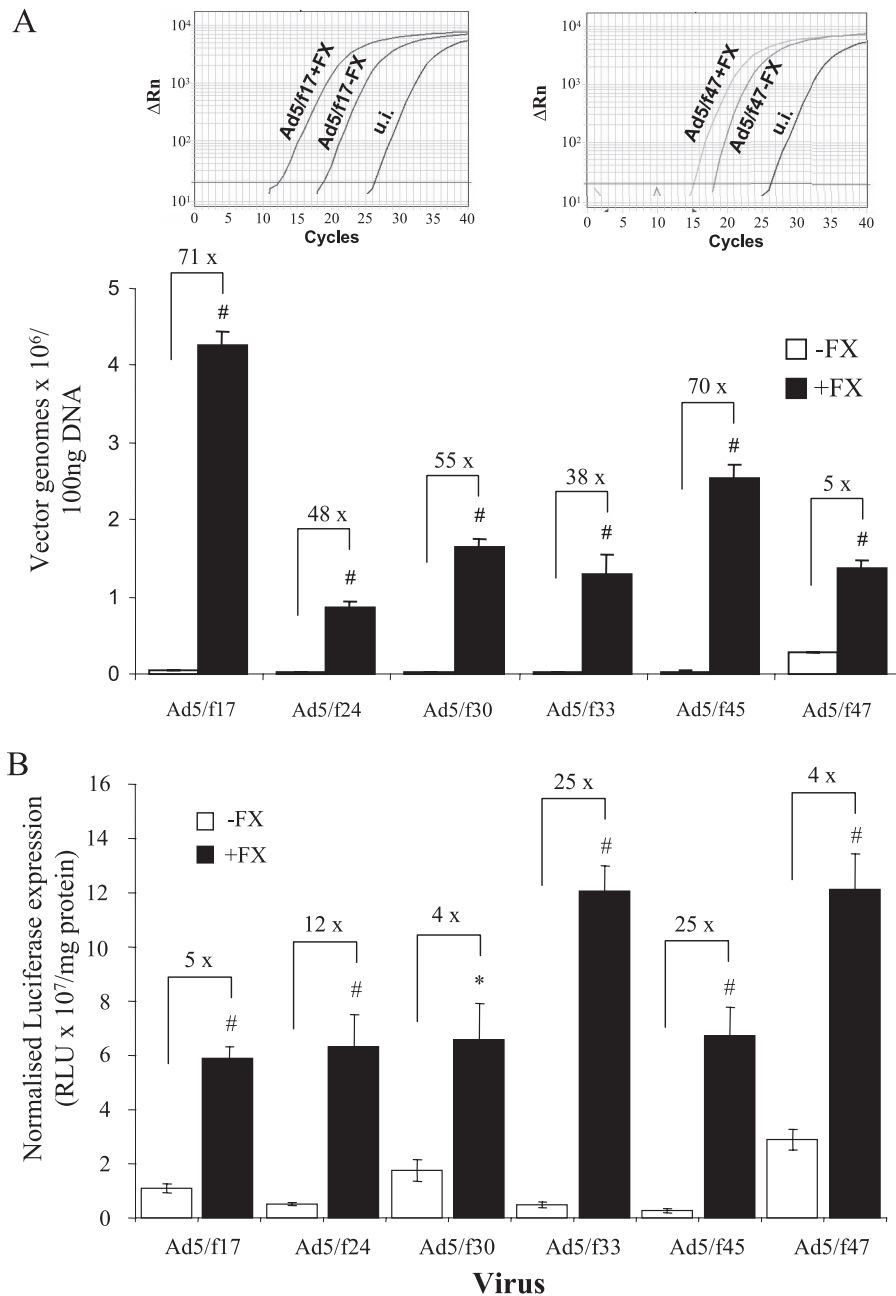


FIG. 2. Effect of physiological FX concentrations on Ad-mediated binding and transduction of HepG2 cells. HepG2 cells were infected with each Ad (1,000 viral particles/cell) in the absence or presence of physiological levels of FX for various times in serum-free medium. (A) Cell binding analysis by TaqMan of recovered viral genomes following exposure to cells at 4°C for 1 h. Representative TaqMan traces are shown for Ad5/f17 and Ad5/f47 in the presence and absence of FX. u.i., uninfected cells; ΔRn , change in normalized fluorescence. (B) Cell transduction by luciferase and total protein quantification at 72 h postinfection following exposure to FX for 3 h at 37°C. *, $P < 0.05$ versus virus in the absence of FX; #, $P < 0.01$ versus virus in the absence of FX (Student's t test). The numbers above the bars represent the changes (n -fold) in comparison with the value in the absence of FX. This figure is representative of three independent experiments. RLU, relative light units.

ing of Ad5-based gene delivery vectors to alternate sites (e.g., disseminated cancers, alternate organs, and specific sites of disease that lack medically achievable surgical access), it is likely that modulation of this pathway will be required, since binding of coagulation factors to the virus is efficient (15). Previous studies have suggested that the fiber is the major determinant of FIX binding to the capsid (16). A simple

method, therefore, to alter susceptibility to coagulation factor binding may be to use fibers derived from alternate serotypes and pseudotyped onto the Ad5 capsid. Shayakhmetov and co-workers demonstrated that Ad5 capsids possessing the fiber of serotype 35 (subgroup B) showed enhancement of cell infection mediated by FIX (16). Subgroup D-based Ads, either as pseudotyped viruses or as complete serotype viruses, are being developed for a variety of clinical applications, including tar-

geted in vivo gene delivery and vaccination (5, 12). Additional virological importance may be assigned to coagulation factor binding, since adenovirus infections are relatively common (and heterogeneous with respect to adenovirus subgroups, including subgroup D) in immunocompromised patients (13). Many of the receptors for adenoviruses from this subgroup remain to be isolated, and those that have been characterized often bind with relatively low affinity (1, 2, 4, 5). Since virus exposure to the blood and hence dissemination may occur in a number of such applications, it is important to assess coagulation factor binding capacity. We documented this capacity by using a panel of luciferase-expressing Ad5 vectors pseudotyped with fibers from subgroup D, including f17, f24, f30, f33, f45, and f47 (see Fig. 1A for a phylogenetic depiction) (7–9).

We first screened viruses for coagulation factor binding by using surface plasmon resonance. We used biosensor chips with immobilized FX or FXI (negative control) (15). All viruses bound to FX but not to FXI (see Fig. 1B for Ad5/f45 as an example). In each case, and consistent with previous data for Ad5 (15), no dissociation of virus from FX was observed until 3 mM EDTA was added, indicating a strong and calcium-dependent interaction with FX (see Fig. 1B for Ad5/f45 as an example). This demonstrates that all viruses evaluated directly bind to FX.

We next sought to determine whether physiological concentrations of FX could influence HepG2 cell binding and transduction mediated by each pseudotyped adenovirus in vitro. Previously we have used this in vitro model as a reproducible model system to examine the effect of coagulation factors on human cell transduction (15). We exposed cells and viruses to physiological concentrations of FX (10 μ g/ml, 1 IU/ml) in serum-free medium for 1 h at 4°C (for cell binding studies) or for 3 h at 37°C followed by incubation for 72 h (for transduction studies). All subgroup D fiber-pseudotyped vectors showed a significant increase in cell binding (Fig. 2A) and subsequent transgene expression (Fig. 2B) in HepG2 cells in the presence of FX, ranging from a 3.7-fold increase in transduction in the presence of FX for serotype Ad5/f30 to a 24.9-fold increase for Ad5/f33 (Fig. 2B). This demonstrates that physiological FX concentrations substantially enhance cell binding and transduction of HepG2 cells mediated by each subgroup D fiber-pseudotyped virus.

To further investigate the effect of other coagulation factors with the γ -carboxylglutamic acid–epidermal growth factor-like (EGF)–EGF–serine protease domain structure on virus transduction, we transduced HepG2 cells with a subset of viruses, Ad5/f47, Ad5/f33, and Ad5/f30, in the presence of physiological concentrations of FVII, FIX, FX, or FXI (negative control) (Fig. 3). All reagents were purified proteins obtained from Hematological Technologies, Ltd., except FVII, which was recombinant (15). All subgroup D-pseudotyped viruses showed a substantial enhancement of HepG2 cell transduction in the presence of FX but not with FVII, FIX, or FXI (Fig. 3). The lack of effect of FVII and FIX was surprising based on previous observations for Ad5 (15, 16) and for the subgroup B Ad35 (16). Although further experiments may be required to assess direct interactions of individual coagulation factors and each virus as well as recombinant forms of FIX, these data do suggest potential subgroup-specific differences in fiber-coagulation factor interactions.

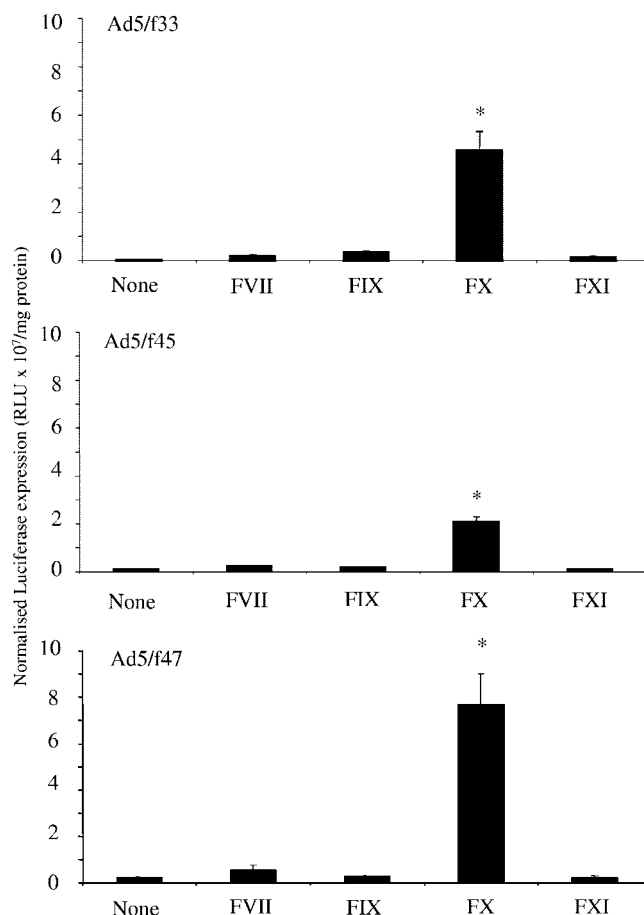


FIG. 3. Effects of FVII, FIX, and FX on transduction of HepG2 cells. HepG2 cells were incubated with 1 IU/ml of human FVII, FIX, or FX or the divergent control zymogen FXI in the absence or presence of each Ad (1,000 viral particles/cell) for 3 h. Transgene expression was quantified at 72 h postinfection and normalized to the total protein content. *, $P < 0.05$ versus virus in the absence of FX; #, $P < 0.01$ versus virus in the absence of FX (Student's t test). This figure is representative of three independent experiments. RLU, relative light units.

We hypothesized that the increase in transduction mediated through FX would be via an interaction with heparan sulfate proteoglycan (HSPG), as observed for Ad5 (15, 16); therefore, CHO cell infections were performed for each virus. CHO-K1 cells (wild type) express HSPGs, whereas CHO-pgsA745 cells have been genetically modified to be deficient in polymerization of HSPGs and therefore have no cell surface expression (6). In each case, physiological levels of FX enhanced transduction in CHO-K1 cells (Fig. 4). However, this level was significantly reduced for CHO-pgsA745 cells (Fig. 4). Therefore, for subgroup D fiber-pseudotyped viruses that show an enhancement in cell binding and transduction through FX, the virus is targeted through HSPG, consistent with the mechanism of retargeting of Ad5 (15, 16). It is noteworthy that the putative HSPG binding site in the third pseudorepeat of the Ad5 fiber (17) is not present in any of the pseudotypes here. However, we recently demonstrated that Ad5 fibers mutated at this site (KKTK motif) show efficient binding to FX (11). Taken together, these data suggest that Ad5 and subgroup D fiber-pseudotyped Ads that are responsive to FX mediate en-

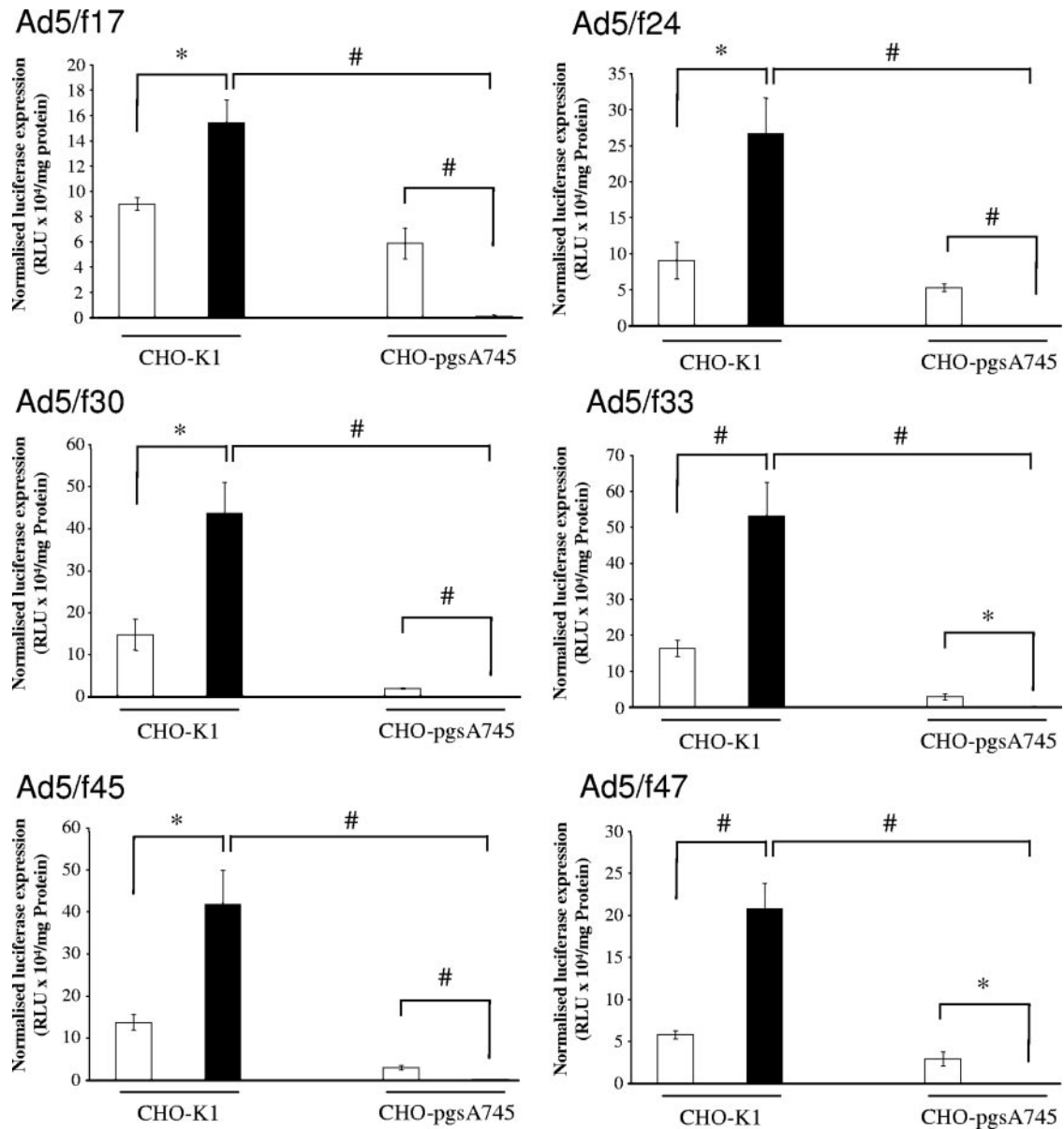


FIG. 4. Effect of FX on virus transduction of CHO-K1 and CHO-pgsA745 cells. CHO-K1 and CHO-pgsA745 cells were infected with 5,000 viral particles/cell of each Ad in the absence or presence of 1 IU/ml FX for 3 h at 37°C, the cells were washed, and the number of transgenes was quantified at 72 h postinfection and normalized to the total protein content. *, $P < 0.05$ versus virus in the absence of FX (Student's t test). This figure is representative of three independent experiments. RLU, relative light units.

hanced cell transduction through retargeting to HSPG on the cell surface. This suggests that coagulation factors play a broad role in dictating adenovirus tropism when exposure to blood occurs, since subgroup C- (15, 16), subgroup B- (16), and now subgroup D-based viruses are all sensitive to FX binding. This is in contrast to a recent finding that Ad5 (and other subgroup C viruses) but not representative viruses from all other subgroups used lactoferrin as a bridge for coxsackie virus and adenovirus receptor-independent transduction of A549 cells (10). This demonstrates that research into coagulation factors and other proteins that can target the Ad capsid proteins in vivo (either following localized exposure in the case of tear fluid and lactoferrin [10] or via exposure to the bloodstream) is

warranted, since this has fundamental implications for receptor utilization and infectivity in vivo.

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