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Chimeric hexon HVRs protein reflects partial function of adenovirus

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ABSTRACT

Adenovirus is widely used in gene therapy and vaccination as a viral vector, and its hypervariable regions (HVRs) on hexon are the main antigen recognition sites of adenovirus. The modification of this area by genetic engineering will change the antigenic specificity of the virus. In addition, recent studies have demonstrated the importance of coagulation factor X (FX) in adenovirus serotype 5-mediated liver transduction in vivo. The binding site of adenovirus to FX is the HVRs on hexon. By constructing five proteins containing chimeric HVRs from different adenovirus serotypes, we focused on the antigenic specificity and the affinity for FX of these proteins compared with the corresponding viruses. Our data showed that HVR5 and HVR7 had only a part of hexon activity to neutralizing antibodies (NAbs) compared with the complete activity of HVR1–7. Results also demonstrated a differential high-affinity interaction of the HVRs proteins with FX and indicated that HVRs protein had a similar binding ability with corresponding adenovirus serotype. These results highlighted some properties of chimeric HVRs proteins and revealed the influence on the structure and function of hexon proteins and adenovirus resulting from the HVRs.

1. Introduction

Recombinant adenoviruses (rAds) have been used as vectors for several potential vaccines. Diseases for which adenovirus vectors are being considered include HIV, tuberculosis, malaria, and cancer [1]. A limitation that has become apparent with Ad5 (best-studied serotype) vaccine vectors is the high titers of Ad5 neutralizing antibodies (NAbs) in human populations, particularly in the developing world [2].

The adenovirus capsid consists of three major structural proteins: hexon, penton, and fiber. Sequence variability among adenovirus serotypes is concentrated on the several loops located at the solvent exposed surface of the hexon, termed hypervariable regions (HVRs). Previous studies showed that the dominant Ad5-specific NAbs are directed primarily against the hexon HVRs [3]. Hexon-chimeric rAd vectors, HVRs exchanges among adenoviruses from different virus subgroups, have been constructed and been proved to evade the majority of pre-existing anti-vector immunity in mice and rhesus monkeys. However, the final yields of these chimeric vectors were still lower than yields of the parental rAd5 vectors and some even have failed to rescue virus [3,4]. The relative importance of the seven individual HVRs as NAb epitopes also remains incompletely understood. Therefore, precisely mapping the NAb epitopes in these seven HVRs, such as HVR5 and HVR7, may be conducive to obtain more perfect chimeric rAd5 vectors [5]. However, construction of large amount of rAd vectors to address this are difficult to operate and expensive. For HVRs, some prior studies also demonstrated that human coagulation factor X (FX) binds the Ad5 hexon via an interaction between the FX Gla domain and HVRs leading to liver infection following intravascular delivery [6]. This specific interaction occurs in multiple (but not all) human adenovirus serotypes and shows diversities in the affinity. Similarly, which domains and amino acids in HVRs are integral to the high-affinity interaction with FX remains unclear [7].

In this study, we constructed and expressed several chimeric HVRs proteins and showed that the native proteins were oligomers and had consistent structure and function with that in virus. These results may provide useful insights for the future development of Ad-based vaccine and gene therapy.

2. Materials and methods

2.1. Cell lines and serum

Human embryonic kidney (HEK) 293, human lung epithelial A549, and human Chang liver cells were obtained from the American Type Culture Collection (ATCC; Manassas, VA). Chang liver cells were cultured in RPMI 1640 medium. HEK293 and

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A549 cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM; Invitrogen, Carlsbad, CA) containing 10% fetal bovine serum, (FBS; Hyclone; Logan, UT) at 37 °C in a humidified 5% CO_2. Anti-Ad5 and anti-Ad37 rabbit polyclonal serums were a gift from Dr. Panyong Mao (Beijing 302 Hospital, Beijing, China).

2.2. HVRs protein expression and purification

Sequences of HVRs from Ad5 and Ad37 were obtained from these two viral DNAs by PCR. The PCR was conducted with primers 5′-GATGAAGCTGCTACTGCTC and 3′-TTCATTTTTATCTGAAAATTCT, 5′-GACTACCAAAGAAAAGCAAAAC and 3′-TTGATTATGGGCTGAAATG, respectively. Sequences of Ad5HVR37(5,7), Ad5HVR37(1–7), Ad5HVR26(5,7), and Ad37HVR5(5,7) containing HVRs exchanged with the corresponding regions from Ad37, Ad26, and Ad5 were produced synthetically (Sangon biotech, shanghai, China). These sequences were then cloned into the pET20b plasmids (Novagen, Madison, WI) respectively by using a BamHI and an XhoI restriction site. Besides, a C-terminal His tag was contained in each of the plasmids. The purified plasmids were expressed in BL21 cells at 18 °C for 12–16 h to produce soluble HVRs proteins. The cells were collected by centrifuging at 4000 rpm for 30 min at 4 °C and resuspended with ice-cold 20 mM imidazole in a buffer containing 25 mM Tris–HCl (pH 8.0) and 500 mM NaCl, and then ultra-sonicated. After centrifugation at 12,000 rpm for 30 min at 4 °C, the supernatant containing HVRs proteins was purified by Ni–NTA beads (Qiagen, Valencia, CA) affinity chromatography. The proteins were eluted with 250 mM imidazole in the same buffer and analyzed by SDS–PAGE.

2.3. SDS–PAGE, gradually-denatured SDS–PAGE (GDS-PAGE) and Native-PAGE

Samples were denatured by boiling at 97 °C in Laemmli buffer (62.5 mM Tris–HCl, 2% SDS, 5% mercaptoethanol, 10% glycerol, 0.002% bromophenol blue) prior to SDS–PAGE. GDS–PAGE was basically similar to SDS–PAGE only except that the samples were not boiled in which the proteins were gradually denatured under the effect of SDS. Native-PAGE differed from SDS–PAGE in that the samples were mixed with a native buffer (62.5 mM Tris–HCl, 40% glycerol, 0.002% bromophenol blue) and were not denatured by boiling prior to electrophoresis performed on 6%–8% Tris–acetate gels (Invitrogen, Carlsbad, CA) without SDS contained in gels and running buffer.

2.4. Western blotting analysis

Protein samples were electrophoresed by SDS–PAGE, GDS–PAGE or Native-PAGE and transferred to nitrocellulose membranes for Western blotting. In particular, SDS was not added into the transfer buffer (39 mM glycine, 48 mM Tris–HCl, 20% methanol, 1.3 mM SDS, pH = 9.2) when proteins experienced Native-PAGE. The rabbit polyclonal serums as primary antibody were used at a 1/100 dilution and the ImmunoPure alkaline phosphatase-conjugated goat anti-rabbit IgG secondary Abs (Jackson ImmunoResearch Laboratories, Inc) were used at a 1/10,000 dilution. The color reaction was performed with 0.1 M Tris–HCl (pH 9.5) containing 0.66% NBT solution and 0.33% BCIP solution.

2.5. Viscotek gel permeation chromatography (GPC) analysis

Protein samples were loaded onto P4000 and P3000 columns using a Viscotek GPC/SEC system (Malvern, Worcestershire, UK) with triple detector platform (LS, RI, and VIS). Bovine serum albumin (BSA, 66 kDa) was used as the standard to calibrate the column. For each analysis, 100 ul of protein sample (1 mg/ml) was loaded and eluted with Dulbecco's Phosphate Buffered Saline (DPBS) at a flowrate of 1 ml/min.

2.6. Circular dichroism (CD) spectroscopy

The conformational integrity of the purified proteins was confirmed by CD spectrum to ensure their natural structures as expected. Far-UV CD spectra were obtained on a Jasco J-810 spectrophotometer (Tokyo, Japan) at 25 °C using a 0.5 cm path length. The solutions containing HVRs proteins were prepared with a concentration of 5 μM in 20 mM phosphate buffer solution (PBS) containing 100 mM NaCl, pH 8.0. The k2d was used to predict the secondary structure content of the proteins for CD spectrum.

2.7. Construction of HVRs chimeric rAd5 vectors

E1/E3-deleted, replication-incompetent rAd5 vectors containing chimeric hexon genes were constructed using a two-plasmid rescue method as described [8]. Partial Ad5 hexon genes containing the Ad5 HVRs exchanged with the corresponding regions from Ad37 or Ad26 were produced synthetically and cloned as ApaI-HpaI fragments into a shuttle plasmid containing the complete Ad5 hexon gene. The Ad5 HVR regions were defined as shown in Fig. 1A. AscI-AscI fragments containing the complete chimeric hexon genes were then excised from the shuttle plasmids and used to replace the corresponding regions in the Ad5 genome plasmid pBHGlox(delta)E1,3Cre. The resultant mutant Ad5 genome plasmids together with the small plasmid pDC316-EGFP expressing enhanced green fluorescent protein (EGFP) or pDC316-Luc expressing luciferase under control of a mouse CMV promoter were co-transfected into HEK293 cells, and homologous recombination yielded rAd5HVR37(5,7), rAd5HVR37(1–7), and rAd5HVR26(5,7) vectors. Plaque purification was performed to isolate a single clone as described [9]. The infectious titer (plaque forming units, PFU/ml) of the rAd vectors was determined by triplicate TCID50 assays using HEK293 cells.

2.8. Protein disrupt Ad5 neutralization assay (NA)

Ad5 NA was assessed by Luc-based virus neutralization assays as described previously [10]. A549 cells were plated at a density of 1×10^4 cells per well in 96-well plates. On the following day, serial dilutions of HVRs proteins with anti-Ad5 polyclonal antibody (Abcam, Cambridge, UK) or Ad5-seropositive rabbit serum were incubated for 1 h at 37 °C in 50 ul reaction volumes. Then a fixed amount of rAd5-Luc reporter constructs at a multiplicity of infection (MOI) of 500 were incubated for 1 h at 37 °C either alone (virus infection alone, VIA), which corresponded to 100% luciferase activity, or with the above protein-serum mixtures. As another positive control for virus replication, only virus with proteins (pAd37HVR) was added to cells, and as a negative control, cells were cultured in the absence of virus and virus with serum. Following 24 h incubation, luciferase activity in the cells was measured using the Steady-Glo Luciferase Reagent System (Promega, Madison, WI) with a Victor 1420 Multilabel Counter (Perkin Elmer, Wellesley, MA).

2.9. Adenovirus infection assay

HEK293 cells were grown in 6-well plates and were infected with the Ad vectors at an MOI of 10 with or without anti-Ad5 and anti-Ad37 rabbit serum. After incubation at 37 °C for 48 h, the fluorescent signal of EGFP was observed by fluorescence microscopy. The signal of the fluorescent proteins was detected at a low magnification (200×) by a fluorescence microscope. For luciferase expression, 2×10^4 Chang liver cells/well were plated

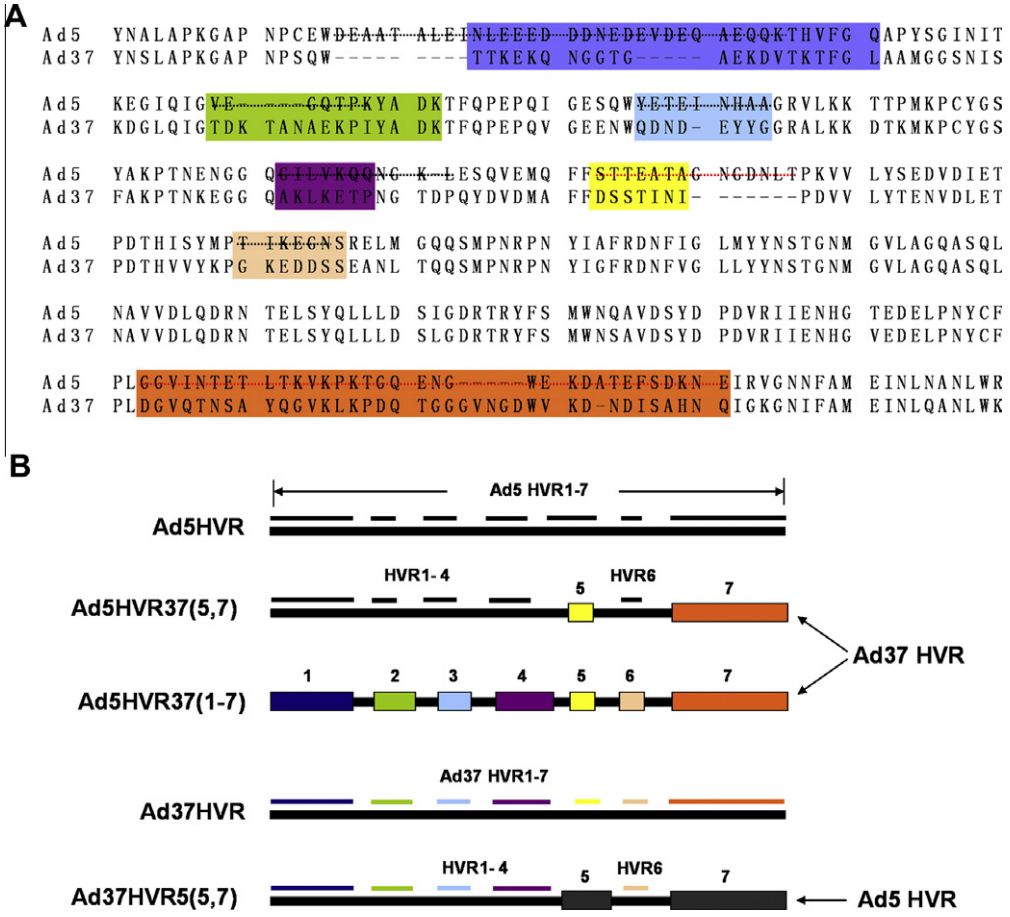

Fig. 1. Sequence and chimeric modification of Ad5 and Ad37 hexon HVRs. (A) Partial sequence alignment showing the residues 121 to 470 of Ad5 and 121 to 457 of Ad37 hexons is indicated by lines. The HVRs to be modified are labeled on the sequence and highlighted in different colors: HVR1 (indigo), HVR2 (green), HVR3 (blue), HVR4 (magenta), HVR5 (yellow), HVR6 (pink), and HVR7 (orange). (B) Schematic cloning strategy to construct Ad5HVR37(5,7), Ad5HVR37(1–7) and Ad37HVR5(5,7) vectors by replacing either the fifth and seventh HVR or all seven HVRs, respectively, with the corresponding regions from each other. The Ad37 HVRs are colored as in panel (A), while the Ad5 HVRs are shown in black. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

into 96-well plates and transferred to serum-free media containing 8 mg/ml FX (Haematologic Technologies Inc). Virus was added at 1000 VP/cell for 3 h at 37 °C. Cells were maintained at 37 °C until harvesting at 72 h. Expression of luciferase was quantified using the Steady-Glo Luciferase Reagent System.

2.10. His Pull-down Assay

His fusion proteins (HVRs) were partially mixed with FX in 500 ul Tris–Ca^{2+} buffer (50 mM Tris–HCl, 150 mM NaCl, 5 mM $CaCl_2$) for 2 h at 4 °C. After incubation, the nickel-nitrilotriacetic acid-agarose metal affinity beads were added for another 2 h binding with gently rocking, followed by centrifugation at 10,000*g* for 10 min at 4 °C and removing the supernatant. Then the beads were washed twice with 500 ul Tris buffer and suspended in 20 ul glycine–HCl pH 2.0 elution buffer. The eluates were mixed with SDS–PAGE loading buffer and separated by SDS–PAGE. His-HVRs and FX were detected in the Western blotting using anti-His and anti-human Factor X (Haematologic Technologies Inc) antibodies, respectively.

2.11. Statistical analysis

Values are expressed as means ± SEM. Differences between groups were examined by Student's t-test (significant when $p < 0.05$).

3. Results

3.1. Construction of chimeric HVR plasmids and analysis of the HVRs proteins

The gene sequences of adenovirus hexon HVRs ranging from HVR1 to HVR7 including conserved regions among each HVR were modified in a different way, and cloned into the pET20b plasmids respectively to obtain the chimeric vectors and express the corresponding proteins (Fig. 1). The native-PAGE analysis indicated that the molecular weight of proteins in solution was over 669 kDa, however, could not be determined precisely (Fig. 2A) even by blue native electrophoresis as described [11] (not shown). As the samples were analyzed by GDS–PAGE in which proteins were gradually denatured, it might be able to speculate the potential forms of polymerization. We observed that trimers or hexamers appeared in some of the samples by Western blotting (Fig. 2B). Viscotek GPC analysis showed that all the HVRs proteins were in an oligomerization state in water solution and the molecular weights were ranged from 4615 to 4820 kDa (Fig. 2C). The CD spectra as shown in Fig. 2D revealed that the secondary structure of chimeric HVRs proteins was slightly changed. Results showed that random coil was a dominant secondary structure (approx. 40%) in the five HVRs proteins which was similar with that in the corresponding virus. Comparing with pAd5HVR, pAd5HVR37(1–7) and pAd37HVR proteins, pAd5HVR37(5,7), and pAd37HVR5(5,7) showed some

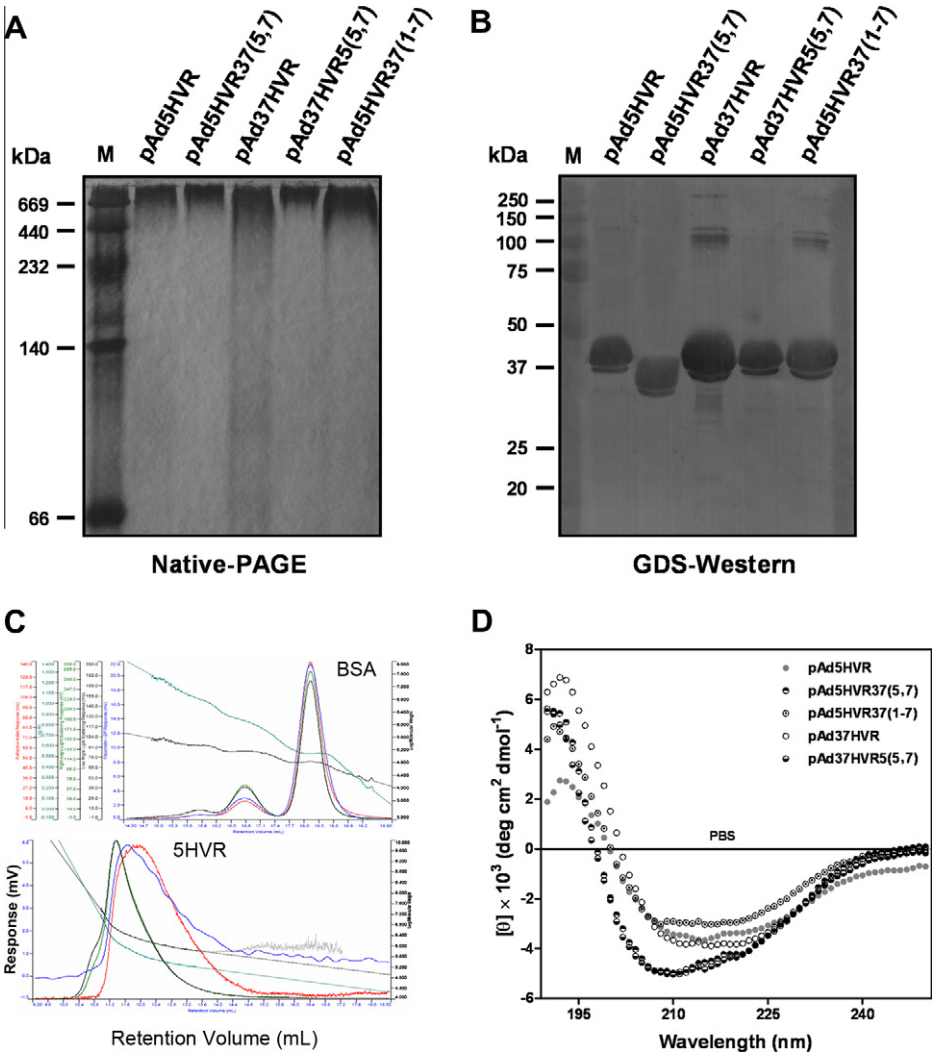

Fig. 2. Molecular weight and structure analysis of HVRs proteins. (A) Native-PAGE of five HVRs proteins to show their molecular weights in solution. High molecular weight markers on the left are indicated. (B) Western blotting of HVR proteins separated by GDS-PAGE. The primary antibody was His mAb (Santa Cruz). Lane M, size standards. The obvious bands around 37 kDa indicate the molecular weights of completely denatured proteins. The upper bands in lane 3 and 5 represent the potential forms of polymerized proteins. (C) Molecular weight of the HVRs proteins were analyzed by Viscotek GPC system. The peak retention volumes (RV, ml) of BSA were 18.123, 16.780, and 15.850 (right to left) which represented molecular weights of 67, 140, and 226 kDa, respectively. 5HVR represented pAd5HVR and its peak RV was 11.823. (D) Analysis of the secondary structure of HVRs proteins in solution using CD. The range of detection wavelength is from 190 to 250 nm. PBS buffer was selected as a baseline control.

changes with increased beta sheet and decreased alpha helix structures. These results indicated the replacement of HVR5 and HVR7 would lead to a certain change in the secondary structure of the proteins, compared with that the replacemen of HVR1–7 would not provide an effect like this.

3.2. Antigenic specificity analysis of chimeric-modified proteins

Anti-Ad5 rabbit serum and anti-Ad37 rabbit serum were used to assess the antigenic specificity of these proteins in native and denatured conditions by Western blotting (Fig. 3A). Under native status, it was found that anti-Ad5 serum recognized pAd5HVR, pAd5HVR37(5,7), and pAd37HVR5(5,7), but the recognition between anti-Ad5 serum and pAd5HVR protein was relatively stronger; anti-Ad37 serum mainly recognized pAd37HVR and pAd5HVR37(1–7) proteins without obvious difference, and pAd37HVR5(5,7) and pAd5HVR37(5,7) proteins could also be recognized by anti-Ad37 serum, but the extent of recognition was somewhat weaker. In denatured condition, no specific band appeared under the effect of anti-Ad5 and anti-Ad37 serum, indicating that anti-hexon antibody preferred to recognize conformational structure of the HVR proteins rather than linear sequence.

To demonstrate that whether the HVRs proteins had the ability to bind NAbs, we performed a Luc-based NA by using HVRs proteins and anti-Ad5 rabbit serum (Fig. 3B). We found that the binding of pAd5HVR protein with anti-Ad5 antibodies resulted in that rAd5, to a large extent, could not be neutralized and exhausted, and in addition, pAd5HVR37(5,7) and pAd37HVR5(5,7) proteins showed a relatively weaker ability to bind NAbs. It was also observed that this binding ability was enhanced with the increased concentration of proteins. It was notable that the percentages of luciferase activity were almost the same when the concentration of pAd5HVR protein was at 25 or 50 μg/ml. This result indicated NAbs might not only react to hexon, but also sparingly bind other capsid components like fiber and penton. However, pAd37HVR and pAd5HVR37(1–7) proteins barely affected the neutralization between anti-Ad5 antibodies and rAd5. It could be perceived that the immunized rabbit serum antibodies mainly presented a specific neutralization to hexon, and the epitope of HVR5,7 had only a part of hexon activity to bind neutralizing antibodies compared with the complete activity of HVR1–7.

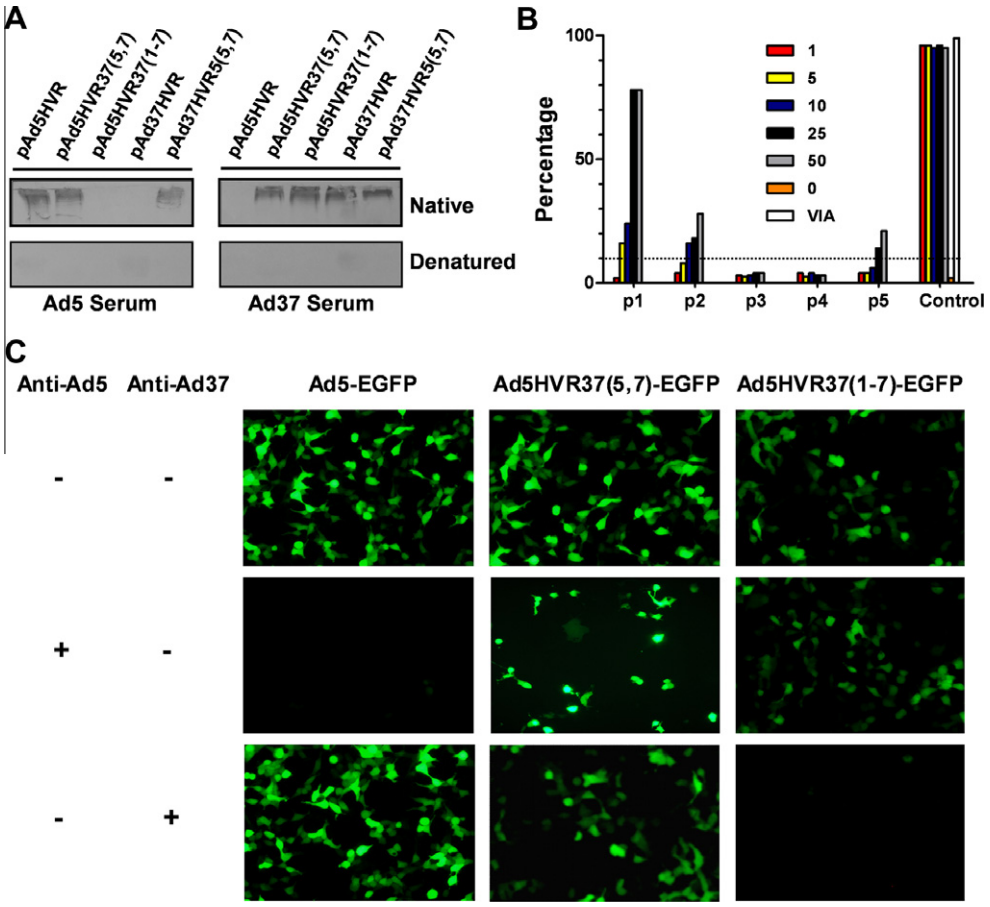

Fig. 3. HVRs chimeric proteins and viruses obtained antigenic specificity from other serotype. (A) Western blotting of the HVR proteins electrophoresed by Native-PAGE or SDS–PAGE and reacted to anti-Ad5 and anti-Ad37 rabbit serum. (B) HVRs proteins disrupt anti-Ad5 serum inhibition of rAd5-Luc infection. rAd5-Luc infecting A549 cells in absence of anti-Ad5 serum (virus infects alone, VIA) corresponds to 100% luciferase activity (MOI = 500). P1–P5 represents pAd5HVR, pAd5HVR37(5,7), pAd5HVR37(1–7), pAd37HVR, and pAd37HVR5(5,7) proteins which were mixed with anti-Ad5 serum and incubated with cells before the addition of rAd5. Number 1/5/10/25/50/0 means the concentration of the proteins (1 unit = 1 μg/ml). In control group (besides VIA), pAd37HVR protein was added with rAd5 without any serum. The dotted line indicated 10% of the maximum luciferase activity as cutoff points. (C) Changes in the immunity of chimeric viruses with replaced HVRs. "Anti-Ad5" and "Anti-Ad37" are serums obtained from rabbits with Ad immunizations and used at a dilution of 1:200. Expression of GFP by rAd infection HEK293 cells were observed under a fluorescence microscope at a magnification of 200×.

3.3. Differences in the ability of HVRs chimeric rAd5 resisting to NAbs

The study of HVRs proteins suggested that they might have similar function with the whole virus. We regarded this as a starting point, and then transformed the corresponding viruses to the similarly chimeric modifications, and used enhanced green fluorescent protein (EGFP) as a signal, to verify that hexon HVRs could be considered as a significant site for virus to evade the NAbs (Fig. 3C). Under the neutralization of anti-Ad5 rabbit serum, rAd5-EGFP was completely blocked, while the rAd5HVR37(5,7)-EGFP and rAd5HVR37(1–7)-EGFP could still infect the cells and express EGFP, although the level of expression was decreased in different extent. In contrast, the presence of anti-Ad37 serum had an markedly influence on the function of Ad5HVR37(1–7) virus, and slightly influenced the expression of rAd5HVR37(5,7)-EGFP. Consequently, HVR5,7 was not a complete neutralizing epitope for Ad5, and the transformation made the virus present a kind of characteristics between the antigenic specificities of these two viruses.

3.4. Human coagulation factor X (FX) binding to chimeric HVRs proteins and rAd5 vectors

We performed a His pull-down assay to investigate the binding possibility between FX and the HVRs proteins (Fig. 4A). It was showed that the binding strength of pAd5HVR protein to FX was higher than that of pAd37HVR, pAd5HVR37(5,7), and pAd5HVR37(1–7), and seemingly similar to that of pAd37HVR5(5,7). pAd5HVR26(5,7) was selected as a negative control because prior study had proved that rAd5HVR26(5,7) virus didn't has affinity with FX [7]. Whereafter, luciferase-tagged rAd5, rAd5HVR37(5,7), and rAd5HVR26(5,7) vectors were used to ananlyze the binding to FX in liver cells compared with the proteins (Fig. 4B). We observed that rAd5HVR37(5,7) had the ability to bind FX but it was actually weaker than rAd5, indicating that this vector obtained a similar binding activity with wild Ad37 [6]. These results showed that the HVRs protein had consistent affinity for FX with hexon and adenovirus serotype. Results also indicated that HVR5,7 was dominant in the high-affinity interaction of HVRs proteins with FX.

4. Discussion

Adenovirus hexon capsomere is a homotrimer of an approximately 900-amino-acid-long polypeptide. X-ray crystallography of the Ad2 hexon trimer [12] has revealed a hexagonal "pedestal" base from which a "tower" region projects outward into the solvent. Three surface loops, L1, L2, and L4, from each monomer interdigitate to form the tower domain. L1 contains six specific regions designated as hypervariable (HVR1–6) and L2 contains the seventh

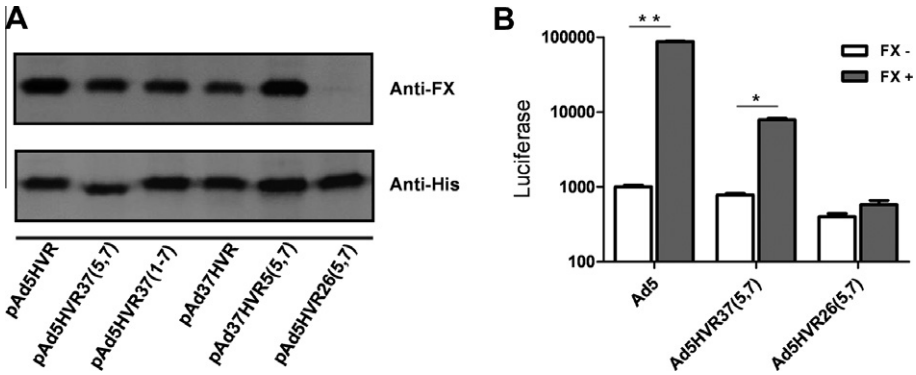

Fig. 4. FX binding to HVRs proteins and HVRs chimeric virus. (A) Proteins incubated with FX and captured by his-tagged beads were separated by SDS–PAGE and analyzed by Western blotting. (B) HVR5,7 chimeric vectors were transfected into human Chang liver cells in or out the presence of FX incubation (represented by gray or colorless columns). $^{**}p < 0.0001$ and $^{*}p < 0.001$.

hypervariable region (HVR7). According to the hexon three-dimensional structure, both of L1 and L2 have seldom beta sheet except for the irregular structures. In our study, CD spectrum showed that the secondary structure of the HVRs proteins were consistent with that in the virus capsid although all HVRs including chimeric protein demonstrated an oligomerization status in native condition, which was similar as a mutant small heat shock protein [13]. HVR5,7 chimeric protein showing some changes in secondary structure indicated that the variable regions in the hexon protein might play a very important role in the trimer structural stability.

The hexon protein of adenovirus contains determinants for both type- and group-specific NAbs [14]. The neutralizing capacity of the Ad5 hexon protein has been associated with the seven HVRs. Prior amino acid homology analysis and structure-based identification of hexon suggested that HVR5 and HVR7 may be candidates for the neutralization epitopes [15,16]. In present study, we showed that native HVRs proteins would recognize the serotype specific NAbs and HVR5,7 contained the Ad-specific NAb epitopes, however, not the key epitopes. Completely modified HVR1–7 would result in a radical change in the antigenic specificity. Construction of the HVRs chimeric rAd5 vectors also indicated that a fraction of Ad5-specific NAbs were directed against HVR5,7.

Previous studies have highlighted the role of a high-affinity interaction of FX with Ad5 hexon in FX-mediated liver gene transfer by Ad5 [6]. The high-affinity interaction of FX with the hexon and cryoelectron microscopy at 23 Å resolution pinpointed the binding to the exposed HVRs [17]. As there were differences in the Ca^{2+}-depended binding between FX and hexon from separated adenovirus serotypes, HVRs chimeric rAd5 vectors would be a meaningful attempt to avoid liver transduction [7]. Data from present study showed that high-affinity interaction of HVRs proteins with FX were consistent with the corresponding viruses as prior study [6,7]. On the other hand, the chimeric HVRs proteins pAd5HVR37(5,7), pAd5HVR26(5,7), and pAd37HVR5(5,7) separately demonstrated similar affinity for FX with the foreign HVR source proteins, indicating that HVR5,7 was the critical amino acids in the Ads (not only Ad5) hexon that bind FX. These results suggested that the HVRs chimeric proteins could also be used to predict the binding activity of the modified adenovirus vectors with FX and guide the construction of novel rAd vectors for gene therapy.

In conclusion, this study highlights the structure and function of hexon HVRs proteins. The structure change of these proteins might be the principal cause for the changes in its immune response and ligand affinity. These results in our study would make sense for the chimeric modification of adenovirus and the future development of Ad-based vaccine and gene therapy.

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References

[1] T.C. Mast, L. Kierstead, S.B. Gupta, A.A. Nikas, E.G. Kallas, V. Novitsky, B. Mbewe, P. Pitisuttithum, M. Schechter, E. Vardas, N.D. Wolfe, M. Aste-Amezaga, D.R. Casimiro, P. Coplan, W.L. Straus, J.W. Shiver, International epidemiology of human pre-existing adenovirus (Ad) type-5, type-6, type-26 and type-36 neutralizing antibodies: correlates of high Ad5 titers and implications for potential HIV vaccine trials, Vaccine 28 (2010) 950–957.

[2] D.H. Barouch, S.V. Kik, G.J. Weverling, R. Dilan, S.L. King, L.F. Maxfield, S. Clark, D. Ng'ang'a, K.L. Brandariz, P. Abbink, F. Sinangil, G. de Bruyn, G.E. Gray, S. Roux, L.-G. Bekker, A. Dilraj, H. Kibuuka, M.L. Robb, N.L. Michael, O. Anzala, P.N. Amornkul, J. Gilmour, J. Hural, S.P. Buchbinder, M.S. Seaman, R. Dolin, L.R. Baden, A. Carville, K.G. Mansfield, M.G. Pau, J. Goudsmit, International seroepidemiology of adenovirus serotypes 5, 26, 35, and 48 in pediatric and adult populations, Vaccine 29 (2011) 5203–5209.

[3] D.M. Roberts, A. Nanda, M.J. Havenga, P. Abbink, D.M. Lynch, B.A. Ewald, J. Liu, A.R. Thorner, P.E. Swanson, D.A. Gorgone, M.A. Lifton, A.A. Lemckert, L. Holterman, B. Chen, A. Dilraj, A. Carville, K.G. Mansfield, J. Goudsmit, D.H. Barouch, Hexon-chimaeric adenovirus serotype 5 vectors circumvent pre-existing anti-vector immunity, Nature 441 (2006) 239–243.

[4] R.R. Bradley, L.F. Maxfield, D.M. Lynch, M.J. Iampietro, E.N. Borducchi, D.H. Barouch, Adenovirus serotype 5-specific neutralizing antibodies target multiple hexon hypervariable regions, J. Virol. 86 (2012) 1267–1272.

[5] S.S. Seregin, A. Amalfitano, Overcoming pre-existing adenovirus immunity by genetic engineering of adenovirus-based vectors, Expert Opin. Biol. Ther. 9 (2009) 1521–1531.

[6] S.N. Waddington, J.H. McVey, D. Bhella, A.L. Parker, K. Barker, H. Atoda, R. Pink, S.M. Buckley, J.A. Greig, L. Denby, J. Custers, T. Morita, I.M. Francischetti, R.Q. Monteiro, D.H. Barouch, N. van Rooijen, C. Napoli, M.J. Havenga, S.A. Nicklin, A.H. Baker, Adenovirus serotype 5 hexon mediates liver gene transfer, Cell 132 (2008) 397–409.

[7] R. Alba, A.C. Bradshaw, A.L. Parker, D. Bhella, S.N. Waddington, S.A. Nicklin, N. van Rooijen, J. Custers, J. Goudsmit, D.H. Barouch, J.H. McVey, A.H. Baker, Identification of coagulation factor (F)X binding sites on the adenovirus serotype 5 hexon: effect of mutagenesis on FX interactions and gene transfer, Blood 114 (2009) 965–971.

[8] P. Ng, R.J. Parks, D.T. Cummings, C.M. Evelegh, F.L. Graham, An enhanced system for construction of adenoviral vectors by the two-plasmid rescue method, Hum. Gene Ther. 11 (2000) 693–699.

[9] P. Ng, R.J. Parks, D.T. Cummings, C.M. Evelegh, U. Sankar, F.L. Graham, A high-efficiency Cre/loxP-based system for construction of adenoviral vectors, Hum. Gene Ther. 10 (1999) 2667–2672.

[10] M.C. Sprangers, W. Lakhai, W. Koudstaal, M. Verhoeven, B.F. Koel, R. Vogels, J. Goudsmit, M.J. Havenga, S. Kostense, Quantifying adenovirus-neutralizing antibodies by luciferase transgene detection: addressing preexisting immunity to vaccine and gene therapy vectors, J. Clin. Microbiol. 41 (2003) 5046–5052.

[11] I. Wittig, T. Beckhaus, Z. Wumaier, M. Karas, H. Schagger, Mass estimation of native proteins by blue native electrophoresis: principles and practical hints, Mol. Cell. Proteomics 9 (2010) 2149–2161.

[12] M.M. Roberts, J.L. White, M.G. Grutter, R.M. Burnett, Three-dimensional structure of the adenovirus major coat protein hexon, Science 232 (1986) 1148–1151.
[13] Z.Z. Wen, Y.H. Wang, B. Yang, M.Q. Xie, K.C. Chou, Roles of L5–7 loop in the structure and chaperone function of SsHSP14.1, Protein Pept. Lett. 18 (2011) 275–281.
[14] C.I. Toogood, J. Crompton, R.T. Hay, Antipeptide antisera define neutralizing epitopes on the adenovirus hexon, J. Gen. Virol. 73 (Pt 6) (1992) 1429–1435.
[15] S. Takeuchi, N. Itoh, E. Uchio, K. Aoki, S. Ohno, Serotyping of adenoviruses on conjunctival scrapings by PCR and sequence analysis, J. Clin. Microbiol. 37 (1999) 1839–1845.
[16] S.L. Pichla-Gollon, M. Drinker, X. Zhou, F. Xue, J.J. Rux, G.P. Gao, J.M. Wilson, H.C. Ertl, R.M. Burnett, J.M. Bergelson, Structure-based identification of a major neutralizing site in an adenovirus hexon, J. Virol. 81 (2007) 1680–1689.
[17] O. Kalyuzhniy, N.C. Di Paolo, M. Silvestry, S.E. Hofherr, M.A. Barry, P.L. Stewart, D.M. Shayakhmetov, Adenovirus serotype 5 hexon is critical for virus infection of hepatocytes in vivo, Proc. Natl. Acad. Sci. USA 105 (2008) 5483–5488.