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Original Article

Sequencing Coding Region and *in Silico* Structural Analysis of Protein Hexon of HAdV-3 Causing Conjunctivitis in Vietnam

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Abstract: Human adenovirus (HAdVs) are responsible for about 65-90% of viral conjunctivitis. Understandings of HAdVs bring promises in prevention, treatments, and biological preparations. However, thorough researches on structural proteins of HAdVs are still limited in Vietnam. In this study, we have sequenced the entire coding gene of protein hexon from HAdV-3 causing conjunctivitis in Vietnam and compared with the reference sequence in NCBI database. We detected 42 DNA variants, of which, 11 resulted in amino acid substitutions. Simulation of HAdV-3 hexon structure showed that all 11 substitutions located in crucial positions. This result revealed high risks from hexon gene variants of HAdV-3 enhancing its life span, virulence, and ability to avoid the host immune system.

Keywords: Conjunctivitis, HAdV-3, Hexon structure

1. Introduction

To date, HAdVs includes roughly 100 different types belonging to family *Adenoviridae*, genera *Mastadenovirus*, which are divided into 7 species represented by letters from A to G [1,2]. The capsid of HAdVs consists of 20 facets made up of 3 main proteins hexon, penton, and fiber. Therein, hexon constitutes the major part of virus coat protein with 12 homo-trimer hexon proteins per facet [3]. The faceted exterior of hexon introduces 7 hypervariable regions (HVRs) in loop-1 and loop-2 structures that consider characteristics for HAdVs genotyping [4,5]. Therefore, researching the structure of hexon protein is important to explore the immune adaptation and develop novel biological applications. Understandings about HAdVs have been worldwide employed in vaccine producing (development) [6] and cancer therapies [7,8]. In Vietnam, there was one research that used HVR-7 to classify different HAdV types associated with conjunctivitis; and HAdV-3

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was one of the types circulating in Hanoi, Vietnam [9]. In this study, we focused on sequencing the coding region and analyzing the 3D structure of hexon protein of HAdV-3 isolated in Vietnam; and then, evaluating the consequences of amino acid substitutions to the life span and virulence of the virus.

2. Materials and methods

2.1 Collecting samples

The eye-swabs of voluntary conjunctivitis patients were collected at Vietnam National Institute of Ophthalmology. The samples were kept in 1.5 ml tubes and stored at -20^{o}C until used for viral DNA extraction.

2.2 Viral DNA extraction and detecting the presence of HAdV-3

The genome of HAdVs was purified by using Viral Gene-Spin Virus RNA/DNA Isolation Kit manufactured by iNtRon company (Korea). Viral DNA elution was stored at -20^{o}C, ready for PCR. Then, we amplified and sequenced the HVR-7 region to detect the presence of HAdV-3 in the samples. The methods were described in more detail by Ha, et al. [9].

2.3 Designing primers for amplifying hexon coding region

We collected and aligned the HAdV-3 genomes available in NCBI database to find the conserved regions flanking hexon gene. The first primer pair HF1-HR1 was designed based on the conserved regions. Then, like the primer walking technique, the second primer pair HF2-HR2 was designed on 3'-terminator region of the first sequences. Similarly, with primer pair HF3-HR3, we could obtain the full coding sequence of hexon gene of HAdV-3 (Table 1)

Table 1. Sequence of primers used to amplify hexon gene of HAdV-3 in Vietnam

Primers	Sequence (5'-3')	Amplicon size (bp)
HF1	CTTAATGACTGTTGACGCTG	4274
HR1	GGATCAAAAAGGTAGCAGGT	
HF2	CTCTGGTATTAACGGCGTAG	3131
HR2	GTATGGATAATTGGCTGGGT	
HF3	GCTTAACTTGCTTGTCTGTG	1878
HR3	TCTGAGGTCATTTCCAAGGG	

2.4 PCR components and thermal cycles

All of the amplifying reactions were carried out with enzyme Phusion Hotstart II DNA Polymerase (Thermo Scientific). Each 50 µl PCR reaction mix contained 10 µl of GC buffer (5X), 1.5 µl of each forward and reverse primer (10pmol µl^{-1}), 1 µl dNTPs mix (10 µM each), 1.5 µl DMSO (100%), 0.5 ul enzyme (2 U/µl) and 1 µl of sample template. Amplifications included 30s initial denaturation at 98°C, followed by 40 cycles at 98°C (20 s), 60°C (10 s), and 72°C (1-3 min), with a final extension at 72°C (5 min). Annealing temperature was optimized for each primer pair and time for an extension of each reaction was calculated based on the speed of polymerase as 30 seconds for each 1 kb. All PCR products were checked by electrophoresis on 1% agarose gel at 90V, 30 min. The gel was then dyed with ethidium bromide and the picture was captured by Alphaimager MINI system.

2.5 DNA sequencing

PCR products with desired bands were sent to 1stBASE company (Malaysia) and sequenced

by BigDye® Terminator v3.1 cycle sequencing kit.

2.6 Analysis of DNA sequence

Sequencing results were read and edited on BioEdit program [10]. The coding sequence of hexon gene of HAdV-3 in Vietnam was aligned with reference sequence NC_011203.1 by ClustalW program [11].

2.7 Structural analysis of hexon protein

The DNA sequence was translated to amino acid sequence (1 letter code) in Snapgene software then align with 9 other hexon protein sequences from 7 different HAdV types. The putative 3D structure of hexon of HAdV-3 in Vietnam was constructed by Swiss-Model program [12], based on its amino acid sequence and data in Protein Data Bank (PDB). The obtained structure was performed on pyMOL software [13].

3. Results and discussion

3.1 PCR amplification of designed primer pairs

The length of hexon gene was 2835 bp. However, the amplicon obtained from PCR with HF1-HR1 primers was 4274 bp long. The desired bands for PCR of HF2-HR2 and HF3-HR3 primer pairs were 3131 bp and 1878 bp. The sequences of primers are described in Table 1. The electrophoresis results in Figure 1 showed that the desired bands were as bright as the marker and no undesired bands were observed. Thus, the PCR products were used for sequencing reactions.

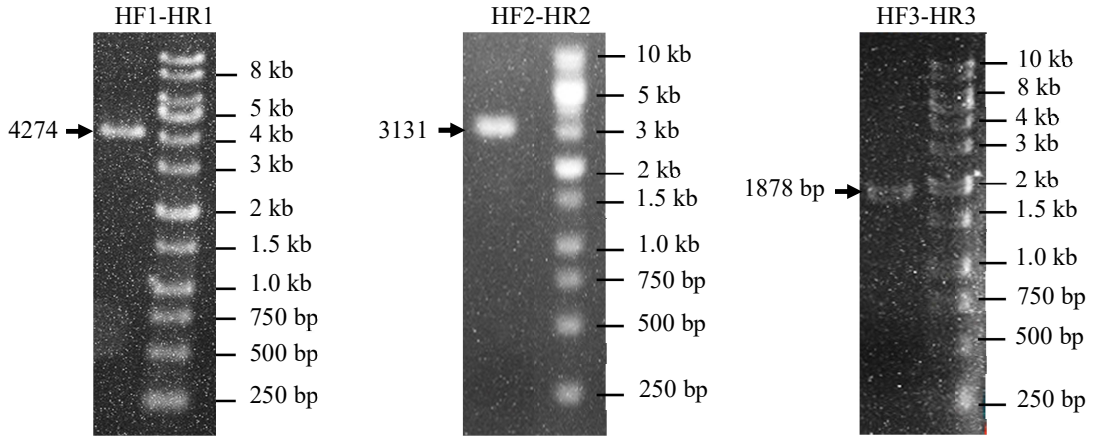

Figure 1. The PCR-amplified products of hexon gene of HAdV-3 in Vietnam with respective primer pairs

3.2 Analysis of DNA sequence

We obtained the full coding sequence of hexon gene of conjunctivitis-induced HAdV-3 in Vietnam. The residual sequences were cut out in BioEdit program. All of the sequencing signals were well resolved with high and clear peaks (Figure 2).

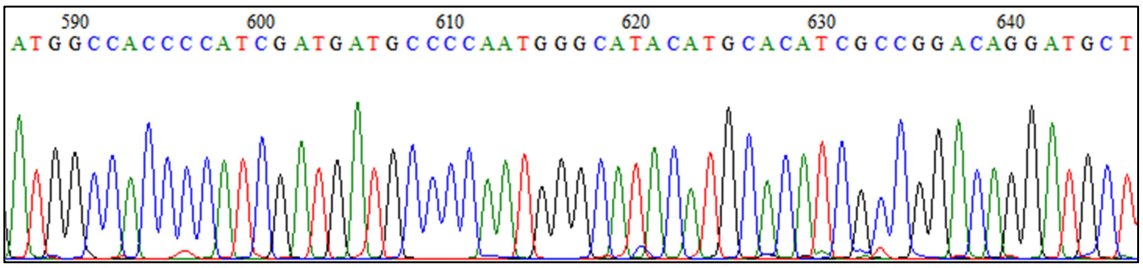

Figure 2. Signals of the first 60 nucleotides of hexon gene of HAdV-3 in Vietnam

We aligned our sequence with the reference sequence of HAdV-3 in the Genbank database (accession number: NC_011203) and detected 42 DNA variants scattering along hexon gene (Table 2), in which, 11 DNA variants led to amino acid substitutions. Nine out of 11 substitutions were observed to locate in hypervariable regions of hexon protein (HVR-1 to 7) [14, 15]. Meanwhile, no variants that cause insertion, deletion, or frameshifts were observed.

Table 2. DNA variants led to amino acid changes in hexon sequence of HAdV-3 in Vietnam

N°	Position	Nucleotide		Amino Acid change	N°	Position	Nucleotide		Amino acid changes
		Seq^1	Seq^2				Seq^1	Seq^2	
1	18489	C	G	-	**22**	20085	G	A	-
2	18542	G	C	G42A	**23**	20103	G	A	-
3	18594	C	T	-	**24**	20149	C	A	-
4	18834	A	G	-	**25**	20202	G	A	-
5	18838	G	C	G141R	**26**	20439	A	G	-
6	18840	G	A	-	**27**	20529	T	C	-
7	19313	A	G	E299G	**28**	20610	T	C	-
8	19321	A	G	N302D	**29**	20694	G	A	-
9	19380	C	A	-	**30**	20712	T	C	-
10	19395	C	T	-	**31**	20856	C	T	-
11	19648	A	G	N411D	**32**	20883	G	A	-
12	19670	C	G	T418R	**33**	20892	A	G	-
13	19671	A	G		**34**	20964	G	A	-
14	19702	A	G	T429A	**35**	20970	C	T	-
15	19733	C	A	A439D	**36**	20976	G	A	-
16	19735	C	A	P440T	**37**	21012	T	A	-
17	19750	A	G	T445A	**38**	21015	C	G	-
18	19758	C	A	-	**39**	21042	G	A	-

19	19818	T	C	-	**40**	21051	C	G	-
20	19929	C	T	-	**41**	21067	A	C	M884L
21	19953	T	C	-	**42**	21084	A	G	-

[1] *Reference sequence of HAdV-3 (NC_011203)*
[2] *The sequence of HAdV-3 sample in Vietnam*
"-" represented no changes of amino acid in this site

3.3 Analysis of protein structure

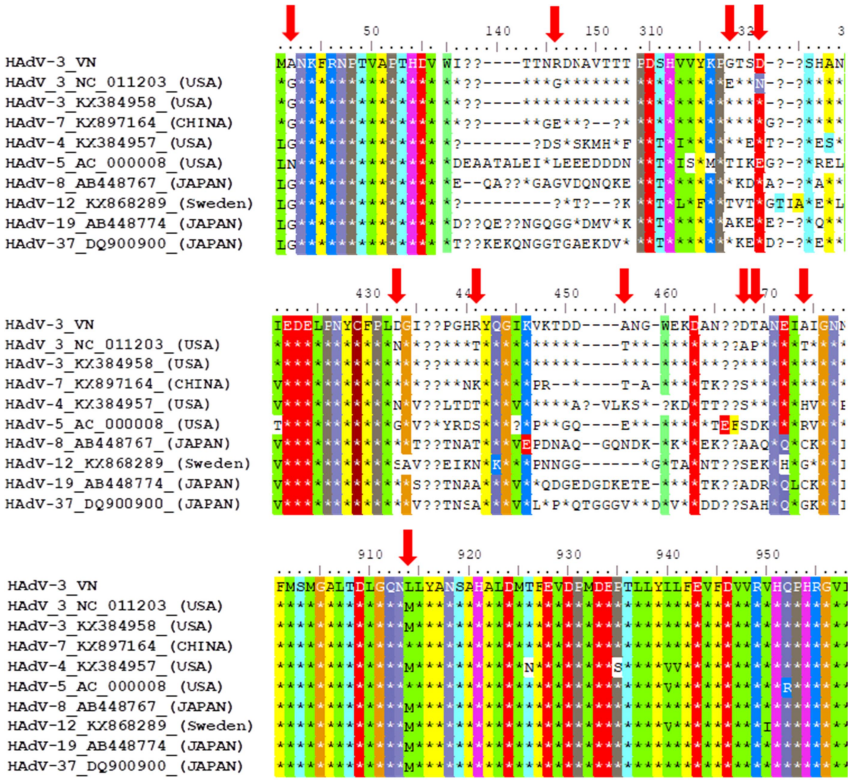

Figure 3. Comparison of hexon protein sequences of HAdVs from different HAdV species

Ten sequences of hexon protein were aligned by ClustalW method using BioEdit software. The result shows the 11 positions of amino acid changes in HAdV-3 VN (red arrow) which compared with type HAdV-3 (NC_01 1203). Most of them belong to HVR regions (no color).

The prior researches showed evidence that the host immune system recognizes and terminates HAdVs through interactions with hexon [18, 19]; we assume, those 9 substitutions on the HVR regions, have high chances of helping the virus avoid the immune activities in Vietnamese patients. Figure 3 showed that most of those substitutions belong to HVR regions. Thus, we predict the 3D structure model of our amino acid sequence to study the meaning of changes for those substitutions.

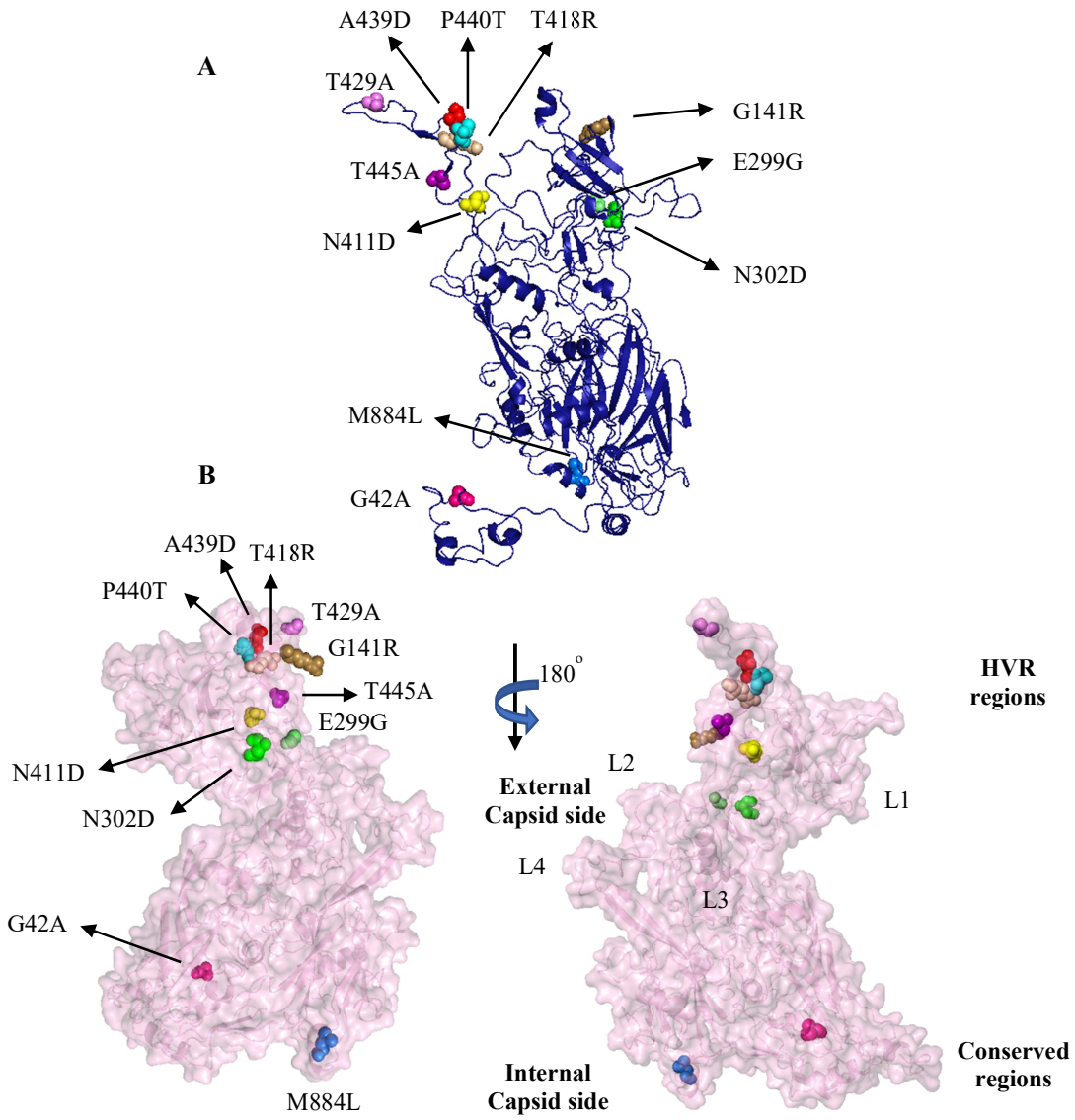

Figure 4. Structure of mono-hexon protein of HAdV-3 VN

Side views of predicted 3D model of HAdV-3 VN mono-hexon protein based on the cryo-EM structure of HAdV-26 (PDB; 5TX1). (A) Cartoon structure of HAdV-3 VN with amino acid substitutions depicted on mono-hexon structure (sideview). (B) The protein surface was represented by 0.65 cartoon transparency. All structure models exposed 11 amino acid changes with highlight by sphere representation as G42A color hot pink, G141R color sand, E299G color lime, N302D color green, N411D color yellow, T418R color wheat, T429A color violet, A439D color red, P440T color cyan, T445A color purple, M884L color marine. L1-14 represent loop 1- loop 4 of hexon protein.

The Swiss-Model program founds 22 structural templates that can be used to build a 3D model of hexon of HAdV-3 in Vietnam. In which, 2OBE [16] has the highest identity to

our sequence (87.11%). However, 2OBE was hexon of Chimpanzee Adenovirus and the protein was described only in the monomer state. Therefore, we chose 5TX1 [17] as the template to build the putative model for hexon of HAdV-3 in Vietnam (identity 84.28%, HAdV-26). All of 11 amino acid substitutions were depicted on putative hexon structure in PyMOL software. Whereby, G42A and M884L located on the inner side (conserved region) of the capsid while the other 9 substitutions were on the outer side (HVR regions) (Figure 4).

Substitution M884L was found in the interacting sites among hexon homo-trimers (Figure 5). Leucine (L) is highly hydrophobic while Methionine (M) is significantly less hydrophobic, which might make bonds among hexon homo-trimers stronger, thus the capsid would be more stable and solid. The last substitution – G42A sited in the central region of each homo-trimer hexon where protein VI clings to (protein VI plays a vital role in cleaving precursor proteins) [20, 21]. Since a neutral amino acid-like Glycine (G) was replaced by Alanine (A) – a hydrophobic amino acid, we suppose the G42A substitution affected the binding ability of protein VI to hexon, stabilized interactions and enhanced virulence of the virus.

Besides, our results showed that the regions forming α helices and β sheets of hexon of HAdV-3 in Vietnam were highly conserved. This agrees with other researches showing that hexon structures of different types: HAdV-26, HAdV-5, and HAdV-2 were almost identical, except HVR regions [17, 22].

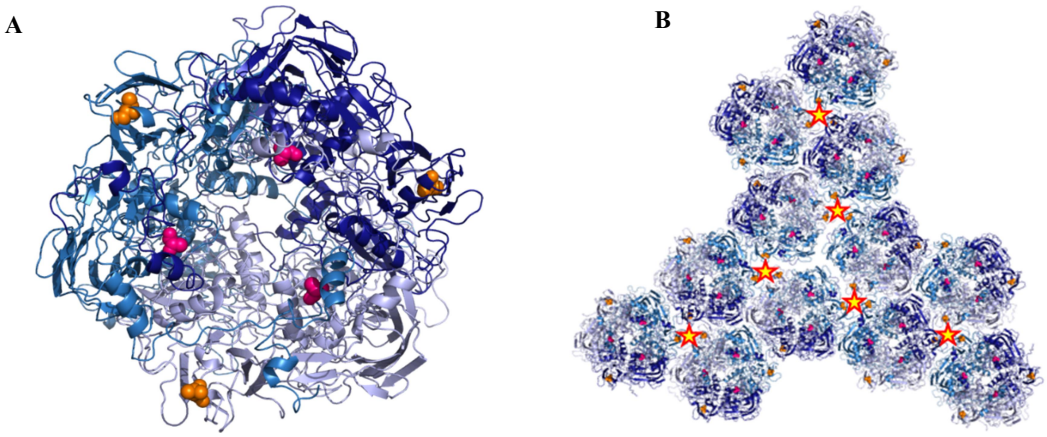

Figure 5. Homo-trimer hexon (view from inside)

A. Substitutions G42A (pink) and M884L (orange). B. 12 homo-trimer hexon proteins make up a facet, M884L substitutions join at "star" signs.

4. Conclusions

We successfully sequenced the coding region of hexon of HAdV-3 causing conjunctivitis in Vietnam. We detected 42 DNA variants throughout the gene, in which, 31 were silent mutations and 11 led to amino acid substitutions. Nine substitutions were determined to locate on HVRs of the capsid, having potentials in helping the virus escaping the immune system. Meanwhile, two substitutions (G42A and M884L) are anticipated to enhance the vitality and virulence of HAdV-3 in Vietnam. Lastly, the putative structure of hexon of HAdV-3 in Vietnam was determined to be conserved with other HAdV types in the world.

Acknowledgments

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