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Short communication

Bioinformatics analysis reveals four major hexon variants of human adenovirus type-3 (HAdV-3) as the potential strains for development of vaccine and siRNA-based therapeutics against HAdV-3 respiratory infections

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ABSTRACT

Human adenovirus type 3 (HAdV-3) encompasses 15–87% of all adenoviral respiratory infections. The significant morbidity and mortality, especially among the neonates and immunosuppressed patients, demand the need for a vaccine or a targeted antiviral against this type. However, due to the existence of multiple hexon variants (3Hv-1 to 3Hv-25), the selection of vaccine strains of HAdV-3 is challenging. This study was designed to evaluate HAdV-3 hexon variants for the selection of potential vaccine candidates and the use of hexon gene as a target for designing siRNA that can be used as a therapy. Based on the data of worldwide distribution, duration of circulation, co-circulation and their percentage among all the variants, 3Hv-1 to 3Hv-4 were categorized as the major hexon variants. Phylogenetic analysis and the percentage of homology in the hypervariable regions followed by multi-sequence alignment, zPicture

HAdV-3 is accountable for 15 to 87% of all adenoviral respiratory infections worldwide with severe morbidity (Kenmoe et al., 2018; Lin et al., 2019; Lynch and Kajon, 2016). Moreover, some strains of HAdV-3 are responsible for 3.6 to 50% fatality among the paediatric age group (Adhikary, 2017; Kajon et al., 1990; Kim et al., 2003; Lai et al., 2013). Therefore, development of a vaccine or a targeted therapy against HAdV-3 is in demand considering its clinical significance.

Hexon protein forms major portion of the HAdV capsid (Russell, 2009). The neutralizing epitopes are located in one or more of the seven hypervariable regions (HVRs) of the hexon (Crawford-Miksza and Schnurr, 1996; Pichla-Gollon, n.d.). Therefore, analysis of HVRs of hexon is extremely important for vaccine strain selection. The worldwide circulating strains of HAdV-3 are categorized into 25 hexon variants (3Hv-1 to 3Hv-25) based on the heterogeneity of the HVRs (Haque et al., 2018), which makes the selection of vaccine strain complicated.

So far, there is no targeted and clinically proven anti-adenoviral, although nucleoside analogues and protease inhibitors showed limited activity (Lion, 2014; Matthes-Martin et al., 2013; Wold et al., 2019). In this regard, synthetic oligonucleotide-based RNA interference (RNAi) provides a tremendous opportunity for the development of oligonucleotide-based drugs (Ge et al., 2003). Among them, small interfering RNAs (siRNAs) has been used as therapeutics against a number of human pathogenic viruses (Ge et al., 2004; Hamasaki et al., 2003; Moore et al., 2005; Zender et al., 2003).

In this study, we have analysed all the hexon variants of HAdV-3 based on various criteria, followed by the molecular analysis of their HVRs to assess their appropriateness as potential vaccine candidates. Then, we have identified the conserved locations in HVR encoding regions of the hexon gene and from those locations we have designed functional siRNAs. Next, we have also designed immunogenic vaccine peptide epitopes from the hexon protein using bioinformatics prediction tool. We hope that our developed siRNAs and immunogenic vaccine peptide epitopes could be used in the future development of siRNA-based therapy and designing a vaccine against HAdV-3.

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vaccine. We anticipate that our developed siRNAs and peptide epitopes could be used in the development of siRNA-based therapy and designing a future vaccine against HAdV-3.

The amino acid (AA) sequences that include the seven HVRs of 25 hexon variants and prototype strain (GB-GenBank accession no. AB330084) were collected from NCBI (http://www.ncbi.nlm.nih.gov/). Hexon variants were selected based on their duration of circulation, co-circulation and worldwide distribution. The selection process is depicted in Fig. 1.

To explore the variations, the 319 AA long sequence (extending from 132 to 450) that included the seven HVRs of the GB strain and 3Hv-1 to 3Hv-4 were aligned by Genetyx software (www.genetyx.co.jp). After alignment the number of AA variations in all the HVRs was observed. Then the differences in AA sequences in all the HVRs were tabulated and the percentage of homologies were calculated manually as compared to the GB strain.

A phylogenetic tree was constructed with the help of the Phylogeny.fr website (http://www.phylogeny.fr/documentation.cgi) (Dereeper et al., 2008) using the “One Click mode”. It has been designed to provide a high-performance platform that transparently chains programs relevant to phylogenetic analysis in a comprehensive and flexible pipeline. By default, the pipeline is already set up to run and connect programs recognized for their accuracy and speed (MUSCLE for multiple alignment and PhyML for phylogeny) to reconstruct a robust phylogenetic tree. The 319AA long (extending from 132 to 450) sequence of 25 hexon variants (3Hv-1 to 3Hv-25) and GB strain were uploaded to the website in FASTA format to build the phylogram.

The variations among the HVR encoding regions of the hexon gene of 3Hv-1 to 3Hv-4 were shown by multi-sequence alignment (MSA), in silico RE analysis (http://rebase.neb.com/rebase/rebase.html) and zPicture analysis (http://zpicture.dcode.org/) (Ovcharenko et al., 2004). For MSA, the 957 bp long (encoding the 7 HVRs) nucleotide (NT) sequences that extend from 394 bp to 1350 bp of the hexon gene of the GB strain and 3Hv-1 to 3Hv-4 were collected from the database of “NCBI” and were aligned using Genetyx software to find out the conserved regions as well as the variation. After identifying the conserved regions, we also aligned the other 21 variants (3Hv-5 to 3Hv-25) to examine whether those same conserved regions exist among the rest of the variants. However, only the alignment data of the GB strain and 3Hv-1 to 3Hv-4 has been shown here as they are the principal variants for analysis in this study. The total number of NT variations on the HVRs among 3Hv-1 to 3Hv-4 were counted manually after the alignment.

In silico RE digestions were performed with BccI, BcoDI, Bsp1286I and BstNI. From the New England Biolab website, REBASE tools were
selected. From the REBASE tools icon, theoretical digest with all the REBASE prototypes icon was selected and opened. Then 957 bp long hexon nucleotide sequences that extend from 394 bp to 1350 bp of the GB strain along with 3Hv-1 to 3Hv-4 were individually pasted in FASTA format for digestion with 1.2% agarose. After digestion, the individual enzyme was selected and the restriction patterns were saved. The 100 bp molecular weight marker (M) was used to detect the molecular weight of the DNA fragments. Then, the restriction profile of BccI, BcoDI, Bsp1286I and BstNI for all the stains were manually arranged side by side for comparison.

Fig. 2. The circulation and co-circulation of four major hexon variants (3Hv-1 to 3Hv-4) based on the available data among seven countries until 2013. 3Hv-1, 3Hv-2, 3Hv-3 and 3Hv-4 were depicted in blue, orange, red and green respectively. We found that the co-circulation of the four major hexon variants was highly prevalent in Korea followed by Germany among those seven countries. In Korea, 3Hv-1 and 2 co-circulated in 1992, and 3Hv-1, 3 and 4 in 1998. 3Hv-1 and 4 again co-circulated in 1999. A single hexon variant also circulated among different countries in the same year, such as 3Hv-1 in Japan, Korea and Taiwan in 1992, and 3Hv-3 in Japan and Germany in 2001. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Fig. 3. Comparison of the AA sequences of seven HVRs of hexon of GB strain and 4 major hexon variants (3Hv-1 to 3Hv-4). The AA sequences of the seven HVRs of GB strain and 3Hv-1 to 3Hv-4 were aligned by Genetyx software. The intervening regions were deleted after alignment. The AA variations as compared to the GB strain at different position of HVRs are indicated by a dot (.) at the bottom and the homologies are represented with an asterisk (*). There were no variations in two HVRs (HVR2 and 5) when compared to the GB sequence. The variations among the rest of the HVRs (HVR1, 3, 4, 6 and 7) in comparison to GB are indicated by highlighting in different colours. The locations of the HVRs in the hexon protein are mentioned in brackets on top.
A comparative study of the HVR encoding regions of the hexon gene of the four HAdV-3 major hexon variants (3Hv-1 to 3Hv-4) with the GB strain was conducted using the zPicture dynamic alignment and visualization tool. For this, the multi-picture option was selected from the website and the 957 bp hexon nucleotide sequences that extend from 394 bp to 1350 bp of HAdV-3 GB strain and 3Hv-1 to 3Hv-4 were uploaded in FASTA format. zPicture compares the nucleotide sequences of pairs of genomes using a moving overlapping window and scoring percent identities. The ‘x’ axis is the size of the nucleotide sequence in kilobases and the ‘y’ axis is the percent identity. The ‘y’ axis was set between 90% and 100%, indicating a very high level of identity. The gapless display blocks indicate homology and the intervening gap regions indicate a lack of homology.

### Table 1
Variations of amino acid (AA) sequences in the HVRs of hexon of 3Hv-1 to 3Hv-4.

| Variant | HVR-1 (132–165) | HVR-2 (171–189) | HVR-3 (197–210) | HVR-4 (233–258) | HVR-5 (265–282) | HVR-6 (299–309) | HVR-7 (411–450) |
|---------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| 3Hv-1   | 141st AA R(G)   | No Variations   | 205th AA V(G)   | No Variations   | No Variations   | 299th AA G(E)   | 322nd AA D(N)   |
|         |                 |                 |                 |                 |                 | 411th AA D(N)   |                 |
|         |                 |                 |                 |                 |                 | 417th AA N(H)   |                 |
|         |                 |                 |                 |                 |                 | 418th AA R(T)   |                 |
|         |                 |                 |                 |                 |                 | 429th AA A(T)   |                 |
|         |                 |                 |                 |                 |                 | 440th AA T(P)   |                 |
|         |                 |                 |                 |                 |                 | 445th AA A(T)   |                 |
|         |                 |                 |                 |                 |                 | 447th AA D(A)   |                 |
|         |                 |                 |                 |                 |                 | 449th AA T(P)   |                 |
|         |                 |                 |                 |                 |                 | 455th AA A(T)   |                 |
| 3Hv-2   | 141st AA R(G)   | No Variations   | No Variations   | No Variations   | No Variations   | 299th AA G(E)   | 322nd AA D(N)   |
|         |                 |                 |                 |                 |                 | 411th AA D(N)   |                 |
|         |                 |                 |                 |                 |                 | 418th AA R(T)   |                 |
|         |                 |                 |                 |                 |                 | 429th AA A(T)   |                 |
|         |                 |                 |                 |                 |                 | 440th AA T(P)   |                 |
|         |                 |                 |                 |                 |                 | 445th AA A(T)   |                 |
|         |                 |                 |                 |                 |                 | 447th AA D(A)   |                 |
|         |                 |                 |                 |                 |                 | 449th AA T(P)   |                 |
|         |                 |                 |                 |                 |                 | 455th AA A(T)   |                 |
| 3Hv-3   | 141st AA R(G)   | No Variations   | No Variations   | No Variations   | No Variations   | 299th AA G(E)   | 322nd AA D(N)   |
|         |                 |                 |                 |                 |                 | 411th AA D(N)   |                 |
|         |                 |                 |                 |                 |                 | 418th AA R(T)   |                 |
|         |                 |                 |                 |                 |                 | 429th AA A(T)   |                 |
|         |                 |                 |                 |                 |                 | 440th AA T(P)   |                 |
|         |                 |                 |                 |                 |                 | 445th AA A(T)   |                 |
|         |                 |                 |                 |                 |                 | 447th AA D(A)   |                 |
|         |                 |                 |                 |                 |                 | 449th AA T(P)   |                 |
|         |                 |                 |                 |                 |                 | 455th AA A(T)   |                 |
| 3Hv-4   | 141st AA R(G)   | No Variations   | 205th AA V(G)   | 254th AA I(T)   | No Variations   | 299th AA G(E)   | 322nd AA D(N)   |
|         |                 |                 |                 |                 |                 | 411th AA D(N)   |                 |
|         |                 |                 |                 |                 |                 | 417th AA N(H)   |                 |
|         |                 |                 |                 |                 |                 | 418th AA R(T)   |                 |
|         |                 |                 |                 |                 |                 | 429th AA A(T)   |                 |
|         |                 |                 |                 |                 |                 | 440th AA T(P)   |                 |
|         |                 |                 |                 |                 |                 | 445th AA A(T)   |                 |

The numbers inside the parentheses in the top row indicate the locations of the HVRs based on the AA sequence of the GB strain. Inside the table, the numbers signify the positions of the AA substitutions and the highlighted letter indicates the name of the AA in an abbreviated form that has substituted the AA present in the GB sequence (as mentioned within brackets).

### Table 2
The percentage of AA homologies in the 7 HVRs of the four major hexon variants of HAdV-3 as compared to the GB strain.

| Variant | Country | HVR-1 | HVR-2 | HVR-3 | HVR-4 | HVR-5 | HVR-6 | HVR-7 |
|---------|---------|-------|-------|-------|-------|-------|-------|-------|
| 3Hv-1   | Korea   | 97.05 | 100   | 92.85 | 100   | 100   | 81.81 | 87.50 |
| 3Hv-1   | Germany | 97.05 | 100   | 92.85 | 100   | 100   | 81.81 | 87.50 |
| 3Hv-1   | Japan   | 97.05 | 100   | 92.85 | 100   | 100   | 81.81 | 87.50 |
| 3Hv-2   | Korea   | 97.05 | 100   | 100   | 100   | 100   | 81.81 | 85   |
| 3Hv-2   | Japan   | 97.05 | 100   | 100   | 100   | 100   | 81.81 | 85   |
| 3Hv-2   | India   | 97.05 | 100   | 100   | 100   | 100   | 81.81 | 85   |
| 3Hv-3   | Taiwan  | 97.05 | 100   | 100   | 100   | 100   | 81.81 | 85   |
| 3Hv-3   | Japan   | 97.05 | 100   | 100   | 100   | 100   | 81.81 | 85   |
| 3Hv-3   | Korea   | 97.05 | 100   | 100   | 100   | 100   | 81.81 | 85   |
| 3Hv-4   | Korea   | 97.05 | 100   | 92.85 | 96.15 | 100   | 81.81 | 87.50 |
| 3Hv-4   | Japan   | 97.05 | 100   | 92.85 | 96.15 | 100   | 81.81 | 87.50 |
| 3Hv-4   | Taiwan  | 97.05 | 100   | 92.85 | 96.15 | 100   | 81.81 | 87.50 |

The GenBank accession nos. are mentioned in the parentheses of each selected hexon variant.

A comparative study of the HVR encoding regions of the hexon gene of the four HAdV-3 major hexon variants (3Hv-1 to 3Hv-4) with the GB strain was conducted using the zPicture dynamic alignment and visualization tool. For this, the multi-picture option was selected from the website and the 957 bp hexon nucleotide sequences that extend from 394 bp to 1350 bp of HAdV-3 GB strain and 3Hv-1 to 3Hv-4 were uploaded in FASTA format. zPicture compares the nucleotide sequences of pairs of genomes using a moving overlapping window and scoring percent identities. The ‘x’ axis is the size of the nucleotide sequence in kilobases and the ‘y’ axis is the percent identity. The ‘y’ axis was set between 90% and 100%, indicating a very high level of identity. The gapless display blocks indicate homology and the intervening gap regions indicate a lack of homology.

Numerous tools are available to design functional siRNAs such as MysiRNA-designer (Mysara et al., 2011), siDirect (Naito et al., 2009) and siMAX-siRNA Designer (https://eurofinsgenomics.eu/en/dna-rna-
In this study, siDirect 2.0 (http://sidirect2.rnai.jp) has been used to design functional siRNAs from the conserved regions found in all hexon variants (3Hv-1 to 3Hv-25). siDirect 2.0 algorithm eliminates off-target effects by reflecting the recent finding that the capability of siRNA to induce off-target effect is highly correlated to the thermodynamic stability, or the melting temperature (Tm), of the seed-target duplex. Hence, the selection of siRNAs with lower seed-target duplex stabilities (benchmark Tm < 21.5 °C) minimizes the off-target effects. It generates and filters siRNAs in three selection steps: step 1 involves the selection of highly functional siRNAs, step 2 involves the reduction of seed-dependent off-target effects and step 3 involves the elimination of near-perfect matched genes (Fookolaee et al., 2019).

We used NetMHC 4.0 (http://www.cbs.dtu.dk/services/NetMHC/) to predict MHC class I binding epitopes. For this, the 319 AA long sequences (extending from 132 to 450) that included the seven HVRs of 3Hv-1 to 3Hv-4 were uploaded in FASTA format. We chose 9mer peptides as most HLA molecules have a strong preference for binding to 9mer peptides. The peptides were identified as a strong binder if the % rank is below the specified threshold for the strong binders, by default 0.5%. On the other hand, the peptide was identified as a weak binder if the % rank is above the threshold of the strong binders but below the specified threshold for the weak binders, by default 2%.

Among the 25 hexon variants, 3Hv-1 to 3Hv-4 comprised 80% of all the variants. We found that they are most prevalent in 7 countries (Japan, Korea, Taiwan, Germany, China, USA and India) as depicted in Fig. 2. Hence, considering the percentage among the variants, global distribution and duration of circulation, we considered 3Hv-1 to 3Hv-4 as the major hexon variants.

The alignment data showed remarkable AA sequence variations among the HVRs of 3Hv-1 to 3Hv-4 as compared to the GB strain. The highest number of 7 variations was found in HVR7 followed by 2 variations in HVR6 (Fig. 3). The locations of the AA substitutions are shown in Table 1. The percentage of homology was found in HVR6 of all the variants. In the remaining HVRs (HVR1, 2, 3, 4, 5, 6 and 7), the percentage of homologies were calculated as 97.05%, 100%, 92.85 to 100%, 96.15 to 100%, and 85 to 87.5% respectively (Table 2).

The different hexon variants (3Hv-1 to 3Hv-25) were segregated into multiple clusters in the phylogenetic tree as shown in Fig. 4. Two of the four major hexon variants (3Hv-1 and 3Hv-4) were incorporated in the same cluster. However, 3Hv-2 and 3Hv-3 were included in a different cluster. Multiple major clusters are formed due to their heterogeneity of the AA sequence in the HVRs.

The alignment data showed remarkable AA sequence variations among the HVRs of 3Hv-1 to 3Hv-4 as compared to the GB strain. The highest number of 7 variations was found in HVR7 followed by 2 variations in HVR6 (Fig. 3). The locations of the AA substitutions are shown in Table 1. The percentage of homology was found in HVR6 of all the variants. In the remaining HVRs (HVR1, 2, 3, 4, 5, 6 and 7), the percentage of homologies were calculated as 97.05%, 100%, 92.85 to 100%, 96.15 to 100%, and 85 to 87.5% respectively (Table 2).

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The NT alignment data of the GB strain and the four major hexon variants of HAdV-3 (3Hv-1 to 3Hv-4) showed a total number of 16 point mutation on the seven HVRs (Fig. 5). There are substantial numbers of conserved regions within the variation in the gene which are depicted in Table 3. The rest of the variants (3Hv-5 to 3Hv-25) also showed similar conserved regions.

In silico DNA restriction patterns with restriction endonuclease BccI, BcoDI, Bsp1286I and BstNI clearly differentiated GB from 3Hv-1 to 3Hv-4 (Fig. 6A). Similarly, the zPicture analysis also clearly depicted the variations (Fig. 6B). We found 10 conserved regions in the HVR encoding portion among all HAdV-3 hexon variants as presented in Table 3. For example, the functional siRNAs designed from the first sequence is shown in Fig. 7. The functional siRNAs designed from the other conserved regions are also shown in the supplementary data 1. However, we could not obtain any functional siRNAs from the conserved region numbers 4 and 6 when the seed-duplex stability (Tm) was < 21.5 °C. We did not try to design functional siRNAs by relaxing the parameters as Tm < 21.5 °C is the minimum requirement for getting off-target reduced functional siRNAs.

MHC class I binding epitopes of 9mer peptides were predicted from NetMHC 4.0. All HLA-A alleles were selected for this. A total number of 311 epitopes were predicted for HLA-A0101. Among them there were 2 strong binders and 5 weak binders based on their % rank as shown in Table 4. The complete dataset of MHC class I binding epitopes prediction from 3Hv-1 has been shown in the supplementary data 2. HAdV-3 respiratory infections have become a global concern, especially among Asian countries (Zou et al., 2012). The only

Fig. 4. Phylogenetic analysis of the partial hexon polypeptide of different hexon variants of HAdV-3. Two strains of 3Hv-1 to 3Hv-4 and one from the rest of the 25 variants were used for this analysis. The hexon variants (3Hv-1 to 3Hv-25) were segregated into multiple clusters. Two of the four major hexon variants (3Hv-1 and 3Hv-4) were included in the same cluster. However, 3Hv-2 and 3Hv-3 were included in different clusters.
The NT alignment data of the GB strain and the four major hexon variants of HAdV-3 (3Hv-1 to 3Hv-4) (GenBank accession nos. AB330084, AF542106, AB067672, KC456085 and AY854180). The total number of variations on the seven HVRs were 16. No variations were found on the encoding regions of HVR2 and HVR5. The NT variations as compared to the GB strain at different position of HVRs are indicated by a dot (.) at the bottom and the homologies are represented with an asterisk (*). Genetyx software was used for the alignment. The encoding regions of the seven HVRs were highlighted in seven different colours and their positions on the hexon gene were mentioned within the brackets.

### Table 3

Conserved regions in the HVR encoding portion among all HAdV-3 hexon variants.

| Number | Positions in hexon gene (GB strain) | Sequence |
|--------|-------------------------------------|----------|
| 1      | 424 bp - 516 bp                     | GACAATGAGTAACCTACACCAACCACAACATTTGCAATGCTCTCTAGTAGCAAGGAGCAGCAATAATCAGTGAATTGGAGTTTTCCGTTAAATGAGGAGAAGAAACCC  |
| 2      | 518 bp - 555 bp                     | TTAACACTGAACGAGGAGAAGAAAAGCCCATTTATGCCATTGAGAAGACGTGCCAGAAATGCTCTAAGCAAGAAGAAACCC  |
| 3      | 571 bp - 613 bp                     | CCAGAGCCTCAAGTTGGAGAAGAATCATGGACTGATACTGATG     |
| 4      | 634 bp - 710 bp                     | GGAAGAGCCCTTAAACCAGCTACCAACATGAAGCCATGCTACGGGTCTTTTGCAAGCTACAAACATAAAAGGGGG  |
| 5      | 714 bp - 740 bp                     | AGCTAAAAACAGAAAAGTAAAACCAAC  |
| 6      | 778 bp - 800 bp                     | GATATGGAATTTTTCGATGGTAG  |
| 7      | 983 bp - 1039 bp                    | GCCTAATGTACTACAACAGTACTGGAAATATGGGAGTTTTGGCTGGCCAAGCATCAC  |
| 8      | 1041 bp - 1096 bp                   | ACTGAATGCAGTGGTTGACTTGCAGGACAGAAATACTGAACTGTCATATCAGCTTT  |
| 9      | 1161 bp - 1211 bp                   | CGATCCCGATGTTCGCATTATTGAAAATCATGGCATCGAGGATGAACTGCC  |
| 10     | 1286 bp - 1315 bp                   | CTAATGGATGGGAAAAAGATGCTAATGCTCGTAGACAGAGGAGTTTTGGCTGGCCAAGCATCAC  |

* GenBank accession no. AB330084
adenovirus live vaccine was developed to prevent HAdV-4 and HAdV-7 acute respiratory infection (ARI) among the military in the United States in 1971. After providing successful protection for a prolonged period, several cases of ARI were observed among vaccinated individuals due to the emergence of mutant strains. Interestingly, analysis of vaccine strains, prototypes and field strains revealed multiple AA changes in the HVRs of hexon (Blasiole et al., 2004; Jacque et al., 2002).

In case of HAdV-3, the selection of vaccine strains is complicated due to the existence of a large number of hexon variants (Haque et al., 2018). In the present study, we have selected 3Hv-1 to 3Hv-4, as they 1) comprise 80% of all hexon variants, 2) have been circulating over longer periods when the circulation of others was shorter and 3) were co-circulating among different countries. Therefore, we have designated them as the major hexon variants. Alignment of the HVR encoding regions of 3Hv-1 to 3Hv-4 revealed 16 point mutations that resulted in 12 AA substitutions when compared to the GB strain. Among them, the lowest percentage of homology was found to be 81.81% which implied 18.19% variation in AA sequence compared to GB. 3Hv-1 to 3Hv-4 were segregated into different clusters in the phylogram because they are sufficiently different from each other and justifies their inclusion as potential candidates for vaccine development.

In this article, we have identified the conserved regions of the HVR encoding genes of 3Hv-1 to 3Hv-4 as well as other variants (3Hv-5 to 3Hv-25) of HAdV-3. From most of those regions, we have succeeded to design functional siRNAs that could be used to inhibit viral replication as there is no targeted anti-adenoviral so far. siRNAs with U, R or A satisfies functional siRNA design algorithms of Ui-Tei et al. (U) (Ui-Tei et al., 2004), Reynolds et al. (R) (Reynolds et al., 2004) and Amarzguioui et al. (A) (Amarzguioui and Prydz, 2004) respectively. Designing siRNAs from the conserved regions to inhibit the viral replication has been used against several pathogenic viruses such as HIV, HCV, HBV, SARS coronaviruses (Hamasaki et al., 2003; Moore et al., 2005; Jacque et al., 2002). After a successful clinical trial, the siRNA-based drug (Onpatro) is now available for the treatment of systemic disease like amyloidosis (Adams et al., 2018). Due to the presence of multiple hexon variants of HAdV-3, we have selected their conserved regions for designing siRNAs, as they will work best against all the variants. siRNA from the conserved region has been successfully used against influenza and HCV (Ge et al., 2003; Ge et al., 2004; Betáková and Svančarová, 2013; ElHefnawi et al., 2016; Tompkins et al., 2004). Hexon protein constitutes 95% of the viral capsomere (Knipe and Howley F Bernard N., 2013). If the hexon gene can be knocked down by siRNAs (Eckstein et al., 2010), the formation of complete viral particles will be prevented and without a complete capsid, HAdV will be unable to infect new host cells (Reynolds et al., 2004). We have also predicted MHC Class I epitopes from the AA sequence of the HVRs (132–450 AA) of each major hexon variant which will save time and cost of future biological work of vaccine development.

Fig. 6. (A) Restriction patterns of GB strain and 3Hv-1 to 3Hv-4 as obtained from the New England Biolabs cutting tool website (http://rebase.neb.com/rebase/rebase.html). In silico restriction endonuclease (RE) digestions were performed with BccI, BcoDI, Bsp1286I and BstNI. The 100 bp molecular weight marker and 1.2% agarose gel were selected for the analysis. (B) Comparative genetics of GB strain and 3Hv-1 to 3Hv-4 using the zPicture pairwise alignment and visualization tool. The nucleotide sequences of the hexon HVRs of the above strains were uploaded in FASTA format on the zPicture web-site (http://zpicture.dcode.org/) for pairwise alignment using the BlastZ alignment program. The ‘x’ axis is the size of the nucleotide sequence in kilobases and the ‘y’ axis is the percent identity. The ‘y’ axis is set between 90% and 100%, indicating a very high level of identity. The gapless display blocks indicate homology and the intervening gap regions indicate a lack of homology.
In this study, we have found that the major hexon variants (3Hv-1 to 3Hv-4) are the most appropriate vaccine candidates against HAdV-3 and the several conserved regions located in the HVR encoding portion of the hexon gene are the suitable sites for designing siRNAs against all the hexon variants. We have designed functional siRNAs from those conserved regions and immunogenic vaccine peptide epitopes from the hexon protein. We expect that our findings could pave the way for the development of vaccine and siRNA-based therapeutics against HAdV-3 respiratory infections.

Table 4
MHC class I binding epitopes as predicted from the AA sequence of the HVR of 3Hv-1 for HLA-A0101. The peptides with a % rank below 0.5 are strong binders and the peptides with a % rank between 0.5 and 2 are weak binders.

| HLA   | Peptide Sequence | Affinity (nM) | % Rank | Bind Level    |
|-------|------------------|---------------|--------|---------------|
| HLA-A0101 | DTDVTNEKF     | 603.54        | 0.40   | Strong Binder |
| HLA-A0101 | YTENVNLET      | 265.81        | 0.30   | Strong Binder |
| HLA-A0101 | ALAPRIVLY      | 3706.52       | 1.30   | Weak Binder   |
| HLA-A0101 | ETPDSHVYY     | 1191.31       | 0.60   | Weak Binder   |
| HLA-A0101 | TSDNVSHNL     | 6941.75       | 2.00   | Weak Binder   |
| HLA-A0101 | NTELSTQLL     | 2808.29       | 1.10   | Weak Binder   |
| HLA-A0101 | LLDSLWDIRT    | 3260.60       | 1.20   | Weak Binder   |

**Declaration of Competing Interest**
None.

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