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Original Article

Nucleotide and Amino Acid Changes in HN, F and SH genes of an Iranian Mumps Virus; RS-12, Following Attenuation to Vaccine Strain

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Abstract

Background and Aims: Wild-type RS-12 strain of mumps virus has been isolated from an Iranian patient and has been attenuated after several serial passages. This study was designed to determine nucleotide and amino acid substitutions in the HN, F and SH genes during attenuation of the wild-type virus.

Materials and Methods: Required viral samples prepared at Razi Vaccine and Serum Institute. Viral RNA was extracted, targeted segments were amplified and sequenced and finally were analyzed using DNAMAN software.

Results: No difference in nucleotide and deduced amino acid sequence of the F and SH genes was detected following attenuation of wild-type as compared to the vaccine strain, but four nucleotide changes in HN gene had occurred which had resulted in two nucleotide differences.

Conclusions: Considering the HN nucleotide sequences and the deduced amino acid sequences, RS-12 wild-type and vaccine strains were distinguishable. Moreover, unique differences between RS-12 and some other vaccine strains existed. During serial passages of RS-12 strain for seed lot system preparation, no change in HN, F and SH genes occurred which -at least in some extents- proved the genetic stability of the attenuated RS-12 surface proteins. Potential attenuating mutations in other genes (N, P, M, L and even non-coding regions in 3′ and 5′ ends) should be investigated and confirmed using advanced methods.

Keywords: RS-12, mumps virus, vaccine.

Introduction

Mumps virus (MuV) is classified as a member of the genus Rubulavirus, within the subfamily of Paramyxovirinae, family of Paramyxoviridae within the order of Mononegavirales. The genome which consists of 15384 nucleotides, is a single strand RNA molecule with negative polarity. The genome encodes seven viral proteins including nucleoprotein (N), phosphoprotein (P), matrix protein (M), fusion protein (F), small hydrophobic protein (SH), haemagglutinin-neuraminidase protein (HN) and viral polymerase (L) (1).

The original wild-type RS-12 mumps virus has been isolated from a throat-washing sample. The isolated virus then has been attenuated after several serial passages in human diploid cells at Razi Vaccine and Serum Research Institute (2).

This study was designed to compare the nucleotide and deduced amino acid sequences of RS-12 surface proteins; HN, F and SH in wild type and attenuated strains to detect the potential mutations responsible for virus

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attenuation. Comparison of some parts of the mentioned sequences with eight well-known vaccine strains and fourteen virulent clinical isolates was also investigated.

Methods

Mumps virus. Wild-type and attenuated RS-12 strains of mumps virus were prepared at human viral vaccines department, Razi Vaccine and Serum Research institute, Iran.
The wild-type virus at passage No.3 and attenuated virus at higher passages as Master seed and as a vaccine seed (different stages of RS-12 seed lot system) were used in this study.
Preparation of viral RNA. High Pure Viral RNA Kit (Roche) was used for extraction of viral RNA from the samples and for cDNA synthesis, 1st Starand cDNA Synthesis Kit For RT-PCR (Roche) was used for synthesis of DNA from extracted viral RNA.
DNA amplification. Taq DNA polymerase was used for setting up the amplification reaction. Following verification of amplification program, pfu polymerase was used to amplify viral cDNA. Since the HN and F genes are relatively long, two pairs of specific primers (which previously had been designed based on highly conserved sequences) were synthesized by MWG (Germany) and used in amplification reactions (3). Since viral SH gene was short in length, amplification with one pair of specific primers were done (Table 1).
Electrophoresis of the PCR products. 1.5% Agarose LE gel was prepared and used. 1Kb ladder, prepared samples and negative control were placed in the wells. Ethidium bromide-stained gels were evaluated for expected bands (Fig. 1). For purification of the PCR products from the gels High Pure PCR Product Purification Kit (Roche) was used and sequencing of purified PCR products was done using MWG instruction. The samples were sent to MWG (Germany) along with the relevant primers for sequencing.
Analysis of sequences. DNAMAN software was hired for assembly and analysis of sequences.

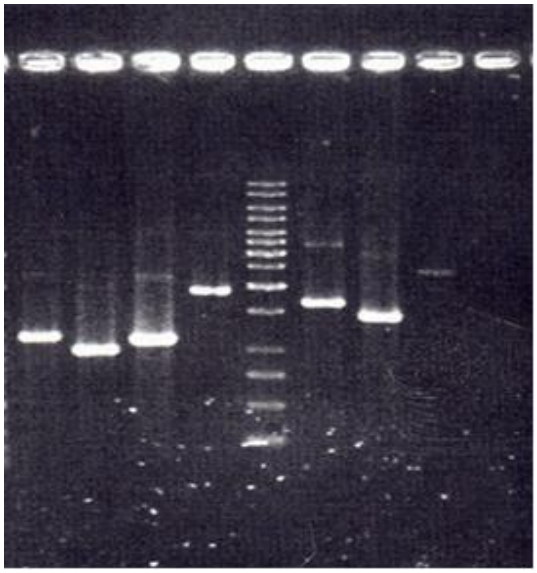

Fig. 1. Electrophoresis of the PCR products. From left to right; SH (1163 nt), F1 (984 nt), F2 (1104 nt), Full length F (1890 nt), 1Kb ladder, HN1 (1668 nt), HN2 (1487 nt), Full length HN (2453 nt), negative control.

Results

A standard seed lot system of Iranian mumps vaccine strain; RS-12, including master, mother, and working seeds have been reported by the author (4). It was an interesting issue to determine what mutations occurred during attenuation of the RS-12 wild-type isolate. Although results have been reported by a colleague on sequence of HN, F and SH genes of the RS-12 wild-type virus, to confirm both previously-published and recent data, amplification and sequencing of the wild-type virus using the same primers was also included in this study.
To prove the accuracy of amplification and sequencing, some measures were considered: (a) pfu polymerase as an enzyme with high fidelity was used, (b) two pairs of specific primers were included to both long genes; HN: 1746 nt and F: 1614 nt, in a manner that there would be two products for each gene with remarkable overlaps with flanking regions, (c) determining the sequence of HN, F and SH genes in four samples that were taken from four serial passages of seed lot system

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preparation. The latter -in some extent- would be a proof of genetic stability during seed preparation as well.

The sequences of HN, F and SH genes of RS-12 vaccine strain were deposited in GenBank under Acc. No. HQ200192 for the first time and the sequences of HN, F and SH genes of wild-type RS-12 strain in this study was completely the same as what previously reported.

There was no difference in nucleotide and amino acid sequences of the four different seeds prepared from attenuated RS-12 strain.

No difference between F nucleotide and amino acid sequences of wild-type and vaccine strains of RS-12 was seen.

No difference between SH nucleotide and amino acid sequences of wild-type and vaccine strains of RS-12 was seen.

Four nucleotides in positions 413, 618, 624 and 653 of HN gene were changed. In all four mutations, nucleotides " T " in wild-type virus were substituted with " C " in attenuated virus. Of these four mutations, T618C and T624C were silent and therefore, resulted in no change in amino acid positions 206 and 208 which were occupied by threonine and serine in both wild-type and vaccine strains, respectively. The other two mutations (T413C and T653C) have resulted in changes as a leucine to proline (L138P) and a valine to alanine (V218A) substitutions in amino acid sequences of wild-type virus.

Multiple Sequence Alignment (MSA) was done on complete nucleotide and amino acid sequences of HN, F and SH genes of RS-12 virus against eight mumps vaccine strains including Jeryl-Lynn, Urabe, L-Zagreb, Smith-Kline Beecham, Chiron, S79, Rubini and Hoshino. There were 34 differences for F gene (Table 2), resulted in 8 amino acid differences in the F protein (Table 3).

For HN gene, 33 nucleotide differences were seen (Table 4) which resulted in 7 amino acid differences in the HN protein (Table 5).

Regarding SH gene, 5 and 4 differences in nucleotide and amino acid sequences (Table 6, Table 7) were seen, respectively.

As a unique difference, the position 138 of HN protein of all mentioned vaccine strains and wild-type RS-12 virus is occupied by leucine which is substituted by proline in RS-12 vaccine strain.

Table 1: Properties of the primers in this study

Gene	Primer name	Sequence	Product name/length
HN	MuHN-6236(+)	GTCGTAACGTCTCGTGAC	HN-1 / 1668
	MuHN-7904(-)	GTTCATACGGCCACCAG	
	MuHN-7202(+)	GTAATTAATGCCAACTGC	HN-2 / 1487
	MuHN-8689(-)	GATCCTTGCAATGAGTTC	
F	MuF-4363(+)	GGAAGTCTGCCTCAATGA	F-1 / 984
	MuF-5347(-)	GCATCTCATCTAGCAGAAC	
	MuF-5149(+)	GAATTAACAACAGTGTTTCAG	F-2 / 1104
	MuF-6253(-)	GTCACGAGACGTTACGAC	
SH	MuSH-5656(+)	GCACTGGATGGAACAATT	SH / 1163
	MuSH-6819(-)	**GACAACTGATTGCTCAAG**	

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There are some evidences that amino acid positions 266, 269, 335, 352, 354, 464, 466 and 498 of HN protein are important in neurovirulence of mumps viruses.

Therefore, a MSA were done with concentration on this positions in wild-type and attenuated RS-12 strains against fourteen virulent clinical isolates which had been deposited in GenBank. The results are summarized in Table 8.

Table 2: Distinguishing differences in F nucleotide sequence of mumps virus, RS-12 vaccine strain, with some other vaccine strains including Jeryl-Lynn, Urabe, L-Zagreb, Smith-Kline Beecham, Chiron, S79, Rubini and Hoshino.

Position	RS-12	Others	Position	RS-12	Others	Position	RS-12	Others	Position	RS-12	Others
33	C	T	348	A	G	696	T	G	1257	C	T
63	C	T	453	A	C	715	G	A	1260	T	C
114	C	A	456	T	C	727	G	T	1378	T	C
178	C	A	480	A	G	753	C	T	1470	T	C
184	G	A	486	C	T	780	A	T	1503	A	G
205	A	G	501	T	A	807	T	G	1509	A	G
209	G	C	519	C	T	886	G	A	1551	G	A
254	G	A	678	C	T	927	T	C			
257	T	C	687	G	A	1227	G	A			

Table 3: Distinguishing differences in F amino acid sequence of mumps virus, RS-12 vaccine strain, with some other vaccine strains including Jeryl-Lynn, Urabe, L-Zagreb, Smith-Kline Beecham, Chiron, S79, Rubini and Hoshino.

Position	RS-12	Others	Position	RS-12	Others	Position	RS-12	Others	Position	RS-12	Others
60	P	T or I	69	I	V	239	A	T	269	I	M
62	D	N	70	S	T	243	A	S	296	V	I

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Table 4: Distinguishing differences in HN nucleotide sequence of mumps virus, RS-12 vaccine strain, with some other vaccine strains including Jeryl-Lynn, Urabe, L-Zagreb, Smith-Kline Beecham, Chiron, S79, Rubini and Hoshino.

Position	RS-12	Others	Position	RS-12	Others	Position	RS-12	Others	Position	RS-12	Others
6	A	G	561	C	T	1044	C	T	1331	T	A
285	G	A	618	C	T	1051	C	T	1395	T	C
342	G	A	684	G	A	1069	C	T	1398	C	T
343	A	C	750	C	T	1080	G	T	1563	A	C
351	G	A	876	T	A	1101	C	A	1567	G	A
374	C	T	924	A	G	1125	G	A	1644	C	T
394	G	A	936	C	A	1207	T	C			
426	C	T	993	G	A	1225	C	A			
468	G	T	1023	T	C	1330	T	C			

Table 5: Distinguishing differences in HN amino acid sequence of mumps virus, RS-12 vaccine strain, with some other vaccine strains including Jeryl-Lynn, Urabe, L-Zagreb, Smith-Kline Beecham, Chiron, S79, Rubini and Hoshino.

Position	RS-12	Others	Position	RS-12	Others	Position	RS-12	Others	Position	RS-12	Others
115	I	L	132	V	I	156	E	D	523	D	N
125	A	V	138	P	L	444	L	Q			

Table 6: Distinguishing differences in SH nucleotide sequence of mumps virus, RS-12 vaccine strain, with some other vaccine strains including Jeryl-Lynn, Urabe, L-Zagreb, Smith-Kline Beecham, Chiron, S79, Rubini and Hoshino.

Position	RS-12	Others	Position	RS-12	Others	Position	RS-12	Others	Position	RS-12	Others
25	C	T	86	C	T	166	C	T			
54	A	C	124	A	G						

Table 7: Distinguishing differences in SH amino acid sequence of mumps virus, RS-12 vaccine strain, with some other vaccine strains including Jeryl-Lynn, Urabe, L-Zagreb, Smith-Kline Beecham, Chiron, S79, Rubini and Hoshino.

Position	RS-12	Others	Position	RS-12	Others	Position	RS-12	Others	Position	RS-12	Others
9	H	Y	29	T	I	42	T	A	56	P	S

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Table 8: MSA of wild-type RS-12 and attenuated RS-12 virus with 14 clinical isolates of mumps virus according to eight position in HN protein with potential importance in viral neurovirulence.

Amino acid position in HN protein	Wild-type RS-12 virus	Attenuated RS-12 virus	14 clinical isolates of mumps virus
266	D	D	D (in 13 cases)
269	A	A	A
335	K	K	K (in 13 cases)
352	G	G	G
354	Q	Q	Q
464	N	N	N (in 13 cases)
466	S	S	S
498	N	N	N

Discussion

Different phenotypes such as plaque size, behavior of virus in cell culture following incubation at specific temperatures, required time for appearance of CPE and the titer of progeny viruses in cell culture have been described as biological markers of attenuation for mumps viruses (2, 5). However, the mechanism of attenuation in different mumps viruses is not the same (6) and it should be determined using more advanced methods, such as reverse genetics and precise assessment of viral virulence in a sensitive and validated animal model (7).

Wild-type RS-12 strain has been isolated from throat-washing of a child with clinical manifestations of mumps disease. The isolated virus has been adapted to cell culture and using further passages in human diploid cell has been attenuated to vaccine strain. Attenuated RS-12 strain has passed all quality control tests including marker tests and neurovirulence test in monkeys (2). Despite this, the mutations during attenuation of this virus had not determined.

Considering the biological properties of HN, F and SH proteins and their important rules in life cycle and infectivity of mumps virus (8, 9 10, 11, 12) this study was designed to determine which mutations have occurred during virus attenuation. In addition, the importance of mutations in HN and F genes on neurovirulence of mumps viruses has been shown by several investigators (6, 13, 14, 15, 16, 17).

A total of 4154 nucleotides including down stream of M gene, intergenic sequence between M and F genes, F gene, intergenic sequence between F and SH genes, SH gene, intergenic sequence between SH and HN genes, HN gene, intergenic sequence between HN and L genes and upstream of L gene were sequenced and analyzed in wild-type and four different passages of the relevant seed lot system.

Among this relatively large sequence, there were only four mutations, which all were in the HN gene. As a consequence, only two mutations have resulted in deduced amino acid substitution in HN protein (L138P and V218A). Considering structural and biological properties of these four amino acids, substitution of Valine with Alanine most probably makes no major effect on HN protein. Although some structural and functional

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changes due to substitution of Leucine with Proline (for instance addition/deletion of a glycosylation site or formation/wrapping an epitope due to conformational changes in HN protein) could be expected, amino acid 138 is not among those motifs that previously have described as functional motifs in HN gene (3, 16). In addition, homology modeling analysis suggested that this mutation has occurred in a relatively variable region of HN protein. However, further experiments such as reverse genetics and evaluation of mutant viruses in a suitable animal model is needed to make any precise conclusion. As a proved example, in Urabe AM9 strain which had been used in vaccine production for many years, position 335 of HN protein determines neurovirulence of the virus. Based on published data, this position is occupied by Arginine and Lysine in the attenuated and neurovirulent viruses, respectively. This strain is no longer used in vaccine production because of frequent viral meningitis due to back mutation in position 335 (5, 6, 15, 17).

MSA of HN amino acid sequence of wild-type and attenuated RS-12 against fourteen clinically virulent isolates (with concentration on eight important positions with potential effect on viral neurovirulence) revealed that almost all positions were occupied by the same amino acids. This finding may suggest the importance of attenuating mutations in other genes; N, P, M, L or even non-coding regions on 3′ and 5′ ends.

Conclusion

Although the nucleotide and deduced amino acid sequences of F and SH in wild-type and attenuated strains of RS-12 virus are the same, there are four nucleotide and two amino acid differences in HN which could be used as a differentiating measure. Furthermore, a unique difference in amino acid position 138 of HN protein found in this study. This position in wild-type RS-12 strain and important vaccine strains (including Jeryl-Lynn, Urabe, L-Zagreb, Smith-Kline Beecham, Chiron, S79, Rubini and Hoshino is occupied by Leucine), but Proline in attenuated RS-12 strain. Beside determination of potential mutations in N, P, M, L genes and non-coding regions, reverse genetic studies and evaluation of neurovirulence test in animal model is necessary for any conclusion on attenuating mutations in RS-12 virus.

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