Sequence Characterization of matrix protein (M1) in influenza A viruses (H1N1, H3N2 and H5N1)

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Abstract

This study brings the analysis of amino acid sequences of matrix protein (M1) from the influenza virus A (H1N1, H3N2 and H5N1) during 2007-2208. 741 sequences of M1 were compared, of them, H1N1 388; H3N2 251 and H5N1 102. Even though, the M1 is relatively conserved among the influenza A viruses, we found some variations in the M1 among the viruses, H1N1, H3N2 and H5N1. The nuclear localization signal at amino acid 101 to 105 is RKLKR for H1N1 and H3N2, but for H5N1 is KKLKR. All differences of amino acid in M1 of H1, H3 and H5 were listed. 80 sequences of M1 of H1N1 H3N2 and H5N1 were used for phylogenetic analysis. There is no reasontant found in the M1 among these subtypes. Further study is needed to study the differences of the function of M1 among H1N1, H3N2 and H5N1. The M1 of H5N1 may contribute to the high pathogenesis to this virus.

Introduction

Influenza virus belongs to the family *Orthomyxoviridae* and possesses a genome of eight single-stranded negative-sense viral RNA that encodes a total of 10 proteins.¹ The membrane protein (M) gene of influenza A viruses is 1027 nucleotides long and encodes two proteins, M1 and M2, derived by splicing of mRNA.² M1 is a matrix protein that lies just beneath the viral envelope in the form of dimers and interact with viral ribonucleoprotein (vRNP). M1 is a relatively small, highly conserved protein, 252 amino acids in type A and 248 amino acid in type B viruses.³ The M1 protein harbors an N-terminal (N) domain (aa 2-67), a middle (M) domain (aa 91-158) and C-terminal (C) domain (aa165-252).⁴ The nuclear location signal (NLS) 95KAVKLYRK-LKR105 is in M domain and C-terminal domain has affinity for RNP.^{5,6} M1 is most abundant protein in virus particle and plays critical roles in many aspects of virus replication. During early viral replication, newly synthesized M1 is transported from its cytoplasmic translation site into the nucleus⁷ via a nuclear localization signal in M1.^{5,8} Later in the replication cycle, accumulation of M1 occurs in the cytoplasm coincident with the export of RNPs from the nucleus⁹⁻¹³ the binding of RNA, nucleoprotein (NP), and M1 is required for the maturation and transport of M1/RNP complex from the nucleus to the cytoplasm.^{14,15} M1 prevents RNP from reentering the nucleus¹³ and M1 also bind to NS2^{16,17} to facilitate nuclear export of the viral RNP¹⁸ in the cytoplasm. M1 interacts with HA, NA, M2 and lipid membrane during budding of new virions form the cell surface.¹⁹⁻²⁵ On the other

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hand, virus budding does not occur in the absence of M1, and M1 expressed along can form virus-like particles in transfected cells.²⁶ Also, Heat shock cognate protein 70 (Hsc70) as a M1 binding protein, the C-terminal domain of M1 interacts with Hsc70. Hsc70 is directly associated with M1, is required for viral production.²⁷

In this paper, we analysis the M1 sequences from the viruses of H1N1, H3N2 and H5N1 downloaded from the gene bank. Even though, M1 is relatively conserved among the viruses, but we have found the variation of M1 among the viruses, H1N1, H3N2 and H5N1. The differences may contribute to the phenotype of these viruses, H1N1, H3N2 and H5N1. Further study is needed to clarify the different functions of M1 of H1N1, H3N2 and H5N1.

Figure 1. Comparison of amino acid sequences of M1 of the H1N1, H3N2 and H5N1 influenza virus isolates. Dots indicate residues identical to the A/Kentucky/UR06-0539/2007.

Table 1. Amino acid differences in M1 among H1N1, H3N2 and H5N1.

Virus	95	101	115	121	137	142	147	160	166	167	168	205	207	218	224	227	230	231	232	239
H1N1	R	R	I	A	A	S	I	K	V	T	T	V	S	A	S	T	K	N	D	A
H3N2	K	R	I	A	A	V	V	R	V	A	T	I	S	A	S	T	R	D	D	T
H5N1	R	K	V	T	T	V	V	R	A	T	I	I	N	T	N	A	R	D	N	A

Materials and Methods

741 of the amino acid sequences of the influenza type A (H1N1, H3N2 and H5N1) M1 were downloaded from Gene Bank, of them, H1N1, 388; H3N2, 251 and H5N1, 102. Multiple amino acid sequence alignment was performed on the prepared set of 3 different subtypes of M1 using the program Bioedit. The phylip format tree output was applied using the bootstrapping procedure; the number of bootstrap trials used was 1000. The tree was drawn with program Tree View.

Results and Discussion

Amino acid sequences of M1 of H1N1, H3N2 and H5N1 have been compared. As shown in Figure 1 and Table 1. The domain of the nuclear localization signal of M1, containing the basic amino acids 101RKLKR105 can bind viral RNA,²⁸⁻³¹ NS2 protein,³² and NP during maturation and preparation of RNPs for export to the cytoplasmic compartment.³³ The basic amino acids of basic region in H5N1 are different from that in H1N1 and H3N2, in H1N1 and H3N2 is 101RKLKR105, but in H5N1 is 101KKLKR105. Hui reported that if R101 changed into A101, the mutant virus yield a lower PFU titer and a smaller plaque size. There is still no report on how about K101 in M1 of H5N1 effects the virus replication.³⁴ The differences of amino acid of M1 of H1N1, H3N2 and H5N1 have been outlined in Table 1. M1 of H5N1 may have different function from that of H1N1 and H3N2, because a single amino acid change in M1 was shown to be responsible for the higher yield in mouse lungs.^{3, 35-38} Fan reported that two amino acid residues in the matrix protein M1 contribute to the virulence difference of H5N1 avian influenza viruses in mice.³⁹ The different amino acid of M1 of H1N1, H3N2 and H5N1 may contribute to the different function of M1. In Figure 2, the polygenetic tree showed that there is no reassortment of M1 happened among subtypes, H1, H3 and H5, although reassortment of human influenza A viruses between the same subtype has occurred frequently.^{40,41} So, the M1 is highly conserved and no reassortment among subtypes. On the other hand, It is possible that M gene determines that host range through

Figure 2. The polygenetic trees of M1 of H1, H3 and H5. Polygenetic trees were from nucleotide sequences by the neighbor-joining method. The scale bar indicates 0.01 nucleotide changes per site. Numbers at the nodes indicate confidence levels of a bootstrap analysis with 1,000 replications as a percentage value.

the interaction between M1 and vRNPs.⁴²⁻⁴⁴ In addition, many studies have shown the interaction between M1 protein and host proteins, such as RACK1, MAPK, and core histone.⁴⁵⁻⁴⁸ The further study is needed to clarify the differences of M1 function among subtypes.

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