Journal of Computational Intelligence in Bioinformatics
ISSN: 0973-385X Volume 3 Number 1 (2010) pp. 91–99

http://www.ripublication.com/jcib.htm

Influenza Virus: Mutational Variants in H5N1 and H1N1

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Abstract

Three major types of influenza virus viz. influenza virus A, influenza virus B and influenza virus C have been reported on the basis of internal antigen (matrix protein which remains bound to ribonucleoprotein of the genome). Influenza virus A was found to have pandemic potential. The 16 H and 9 N subtypes of it were determined on the basis of surface antigens (hemagglutinin and neuraminidase). The H5N1 genome was found to have eight segments of ribonucleoprotein of ssRNA. Each segment codes different protein. A major problem is faced in vaccine development against surface antigens because the virus has very rapid mutation rate. The PB2 gene of segment 1 seems to give an insight to develop a peptide based vaccine for the treatment of bird flu because of a significant mutation at 627 position which has changed the Lys (K) of H1N1 to Glu (E) in H5N1 i.e. K627E mutation.

Key Words: H5N1, H1N1, Ribonucleoprotein genome, K627E mutation.

Introduction

Influenza an acute infectious disease is commonly seen in local outbreaks and epidemics throughout the world with little or no warning. Epidemics may be short lived lasting from few days to few weeks or large lasting for months [15]. In twentieth century three overwhelming pandemics viz. Spanish flu affecting a population of two millions, Asian flu and Hong Kong flu affecting a population of 1 million each were observed in 1918, 1952 and 1968, respectively.

Bird flu (also known as avian influenza, avian flu, Virus A influenza, type A flu or genus A flu) is caused by influenza virus A that is hosted by birds. It may infect

several species of mammals. It was first reported in Italy in the early 1900s and is now known to exist worldwide. The first known appearance of this type of influenza in human was in Hong Kong during 1997 [1]. The infection of humans coincided with an epidemic of avian influenza, caused by the same strain, in Hong Kong's poultry population. The outbreak was stopped by the killing of the entire domestic poultry population within the territory. The outbreak was stopped by killing the entire domestic poultry population within the territory.

Normally, avian flu viruses are transported worldwide in the intestines of wild birds, and are non-lethal. The non lethal variant has mutated into the most lethal and highly pathogenic strain viz. H5N1of avian influenza/ bird flu ever recorded. Infected birds pass on H5N1 through their saliva, nasal secretions, and feces. Other birds may pick up the virus through direct contact with these excretions or when they have contact with surfaces contaminated with these materials. Because migratory birds are among the carriers of the H5N1 virus, it may spread to all parts of the world. Past outbreaks of avian flu have often originated in crowded conditions in southeast and east Asia, where humans, pigs, and poultry live in close quarters. In these conditions a virus can mutate into a form that more easily infects humans. Such mutations are natural and have happened in the past e.g. Spanish flu was caused by a variant H1N1 in 1918.

Human influenza viruses differ from other avian influenza viruses on the choice of cellular receptors. Avian influenza viruses bind to cell-surface glycoproteins or glycolipids containing terminal sialylgalactosyl residues linked by 2-3-linkage [Neu5Ac (2-3)Gal], whereas human viruses, including the earliest available isolates from the 1957 and 1968 pandemics, bind to receptors that contain terminal 2-6-linked sialylgalactosyl moieties [Neu5Ac(2-6)Gal]. Recent evidences suggests that human bronchial ciliated epithelial cells contain Neu5Ac (2-3)Gal and can be infected with avian influenza viruses [14]. Nevertheless, avian influenza viruses can not infect non-ciliated bronchial epithelial cells. Hence, adaptation of the avian influenza virus to non ciliated cells is a prerequisite for a pandemic virus to emerge. Biological behaviours of influenza viruses indicate that once a pandemic virus emerges, isolation is not likely to contain this epidemic [15].

There is a major problem in vaccine development using an approach of developing vaccine against the surface antigens present on virus envelop because the virus has a very rapid mutation rate and modify the genes coding for these proteins/ antigens forcing commercial producers to wait for mutations to occur before developing effective new versions of standard vaccines. A specific vaccine against the pandemic strain will not be available until 6 to 12 months after the inception of the pandemic. Judicious use of antiviral agents and stringent disease control measures are imperative to decrease the impact of a future pandemic [14].

In the present research paper an effort has been made to study the various aspects such as biochemical perspective, mode of infection and mutational analysis of influenza virus. This work may be significant so as to develop an effective vaccine for this deadly infectious disease.

Material and Methods

Protein and Nucleotide Sequence

Almost all the informations including the sequence for the present study were taken from GenBank (NCBI). The Protein sequence was predicted from Primary Protein Sequence database Swiss-Prot. The accession numbers of the sequences used for H5N1 and H1N1 are AF144300 and ABA55038, respectively (from GenBank).

Bioinformatics Analysis

Domains were predicted from Prodom Tool. Prodom is a comprehensive set of protein domain families. Pair wise alignment is done by ALIGN tool. Structural studies were carried out through RASMOL, Swiss-Prodom database Viewer, and Mercury software, etc. Self-optimized prediction method (SOPMA) was used to improve the success rate in the prediction of the secondary structure of proteins.

Results and Discussion

Structure and Biochemical Perspective of Influenza virus:

The influenza virus, a member of the Orthomyxoviridae family has an eight segmented ribonucleoprotein genome of single stranded RNA (ssRNA). As shown in **Figure 1** the complete genome of H5N1 is found to have eight segments of ribonucleoprotein strand of different sizes because of having different number of nucleotides (865bp – 2341bp). Each segment of the genome has genes/ codes for a different protein. The various proteins coded by H5N1 genome are shown in **Table 1.**

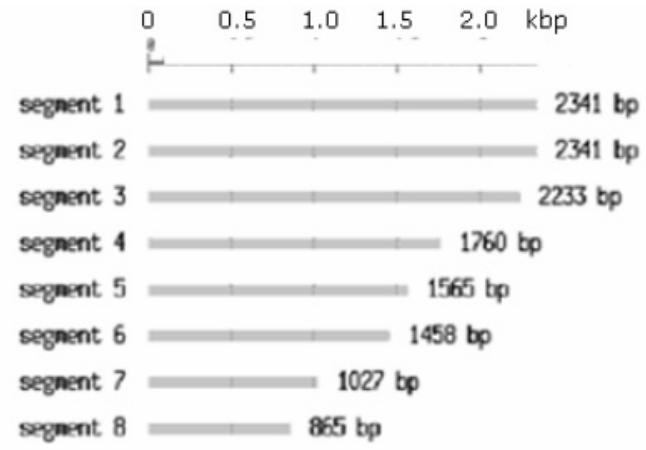

Figure 1: Influenza virus- ssRNA strand (Complete genome Taxonomy id: 93838. Lineage: Influenza A virus (A/Goose/Guangdong/1/96); H5N1 subtype)

Table 1: Various Proteins Coded by H5N1 Genome.

Segment No.	Protein	Gene Name
Segment 1	Polymerase basic protein 2	PB2
Segment 2	Polymerase	PB1
Segment 3	Polymerase	None
Segment 4	Hemagglutanin (H)	None
Segment 5	Nucleocapsid protein	None
Segment 6	Neuraminidase (N)	NA
Segment 7	Matrix protein 2	M
Segment 8	Non structural protein	None

The 8 segmented ssRNA remain enclosed in a lipid bilayer envelop inside of which is lined with matrix protein. The matrix protein remains chemically bound to the ribonucleoprotein to form the internal type specific antigens and are used to differentiate the different types of influenza virus [2]. The gene M on segment 7 codes for the matrix protein. Three major types (A, B and C) of influenza virus have been reported so far. Among these, influenza virus A has the pandemic potential. The various subtypes/ strains of influenza virus are determined by a trimeric protein, hemagglutinin (H) and a box shaped protein, neuraminidase (N) which form the surface antigens and are present as protruding spikes on the surface of virus capsid.

In order to determine how efficiently the polymerase proteins derived from human and avian influenza A viruses can interact with each other in the context of a mammalian cell, a genetic system that allows the *in vivo* reconstitution of active ribonucleoproteins was used. The ability to achieve replication of a viral-like reporter RNA in COS-1 cells was examined with heterospecific mixtures of the core proteins (PB1, PB2, PA and NP) from two strains of human viruses (A/Puerto Rico/8/34 and A/Victoria/3/75), two strains of avian viruses (A/Mallard/NY/6750/78 and A/FPV/-Rostock/34), and a strain of avian origin (A/Hong Kong/156/97) that was isolated from the first human case of H5N1 influenza in Hong Kong in 1997 [1]. Besides enormous economic losses to the poultry industry, recent H5N1 highly pathogenic avian influenza viruses (HPAIVs) originating in eastern Asia have posed serious threats to public health. Up to April 17, 2008, 381 human cases had been confirmed with a mortality of more than 60% [16]. The viruses pose a threat of emergence of a global pandemic influenza through point mutation or reassortment leading to a strain that can effectively transmit among humans [7].

Mutational Variants of Influenza Virus A

There are two models of mutations associated with influenza virus, namely antigenic shift and antigenic drift. The major primary cause of pandemics is antigenic shift which results from the reassortment of the segmented genetic materials between different subtypes of viruses and can transform an influenza virus into a new subtype. The encoding of H and N by separate RNA molecules present on segment 4 and segment 6 (**Table 1**) may facilitate the reassortment of these genes in animals including in humans simultaneously infected by two different subtypes. All the eight segments may take part in the reassortment resulting in the formation of such a unique strain with new mixed genomes altogether different from the parental strains and for which the immune system has to start all over to make new antibodies to combat it.

Antigenic drift results from minor gene mutations and lead to a new strain instead of a new subtype. Since, RNA replication tends to have more errors than DNA replication, therefore, a high mutation rate is there with gradual accumulation of new epitopes on H and N molecules of flu viruses. Thus they drift so much that the original antibody can no longer bind to it giving the viruses a selective advantage. This antigenic drift is responsible for the localized outbreaks of different strains of influenza viruses, especially influenza virus B. Because of these characteristics influenza viruses are capable of causing repeated epidemics in human populations and even pandemics. These interpretations are in accordance with the findings of Nicholson et al. [9].

So far 16 H and 9 N subtypes of influenza A virus have been reported which have been confirmed in apparently healthy free ranging avian species, including waterfowl which are thought to be natural reservoirs of the influenza A virus [13]. The16 types of hemagglutinin and 9 types of neuraminidase have been reported to be present in 16 H (H1 – H16) subtypes and 9 N (N1 – N9) subtypes, respectively. The Avian Influenza A virus (AIV) subtypes H3, H4, H6 are most frequently isolated whereas the AIV of the subtypes H5 and H7 were less frequently encountered. All other H sub types are rather rare. Similarly AIV subtypes N1, N2, N3 and N8 are frequent and all other N sub types are rarely detected [6]. Total 144 combinations of H and N are possible, out of which 103 have been confirmed. The four combinations of H and N viz. H5N1, H7N3, H7N7, and H9N2 are known to have caused human infection.

Mode of Infection and Vaccines Development

It has been reported that H functions during attachment of virus to the sialic acid linked galactose receptor of the host cell membrane i.e. it mediates receptor binding and membrane fusion, thus initiates the infection. Once virus binds to the cell surface, N digests sialic acid (neuraminic acid) which most cells have on their surface and the virus gets endocytosed. Removal of sialic acid of infected host cells surfaces makes it easy for the progeny virons to diffuse away on exiting the cell and thus N facilitates cleavage of the viral progeny from infected cells, prevents viral aggregation, and aids movement of the virus through the mucosal respiratory epithelium.

Ilyushina NA studied the effects of a neuraminidase inhibitor (oseltamivir) and an inhibitor of influenza virus polymerases (ribavirin) against two highly pathogenic H5N1 influenza viruses. During the research clear differences were observed between

the efficacies of the drug combinations against two H5N1 viruses: higher doses were required for the protection of mice against A/Turkey/15/06 virus than for the protection of mice against A/Vietnam/1203/04 virus [5]. The preliminary results suggest that oseltamivir-ribavirin combinations can have a greater or lesser antiviral effect than monotherapy, depending on the H5N1 virus and the concentrations used. The neuraminidase of influenza viruses is the target of the inhibitors oseltamivir and zanamivir. Recent reports on influenza viruses with reduced susceptibility to neuraminidase inhibitors (NAI) are a cause for concern. Several amino acid substitutions, each as a consequence of one single nucleotide mutation, are known to confer resistance to NAI.

Vaccines for avian influenza typically are aimed at hemagglutinin or neuraminidase on the outside of the virus capsid because antibody to H can neutralize the virus preventing infection initiation by not allowing the binding with host cell and antibody to N has the protective effects as it seems to slow the spread of the virus. A major problem with such an approach is that the genes coding for these proteins (H and N) have a very rapid mutation rate, forcing commercial producers to wait for mutations to occur before developing effective new versions of standard vaccines [3, 5].

It has been observed that PB2 gene present on the segment 1 (the longest segment with a polynucleotide of 2341 bases) codes for polymerase basic protein 2 (**Table 1**). Lysine (K) at 627 position of this protein in H1N1 (1918) has mutated to Glutamic acid (E) in H5N1 **(Figure 2)**. H5N1 is known to be pandemic for humans. This finding has resulted in a new approach of vaccine treatment for bird flu. One more mutation at 628 position of PB2 gene which changed Glutamine (Q) in H1N1 to Proline (P) in H5N1 has been observed (**Table 2**), but it was found to have no significant effect on the protein function [2].

Figure 2: The Protein Structure of PB2 Gene of H5N1 genome (Polymerase basic protein 2 showing a mutation at 627 position).

Table 2: Mutated Region in PB2 Gene.

H1N1 PB2 Gene							
Amino Acid	Ala | 623	Ala	Pro	Pro	Lys : :	Glu : :	Ser | 629
H5N1 PB2 Gene							
Amino Acid	Ala | 623	Ala	Pro	Pro	Glu	Pro	Ser | 629

Since segment 1 of genome is having an important gene PB2, therefore, an effort has been made to study its prodom domains and also the secondary structure of its proteins in H5N1 and H1N1. It has been found that the segment 1 of the genome has two domains i.e. Prodom Domain ID: PD001667 and PD217887. The 627 position has been observed to be present on domain PD001667 (**Table 3**).

Table 3: Prodom Domains and Gene Function of Segment 1.

Domain ID	Begin at (Base number)	End at (Base number)	Gene Function
PD001667	11	759	Polymerase PB2 Subunit basic RNA RNA-directed transferase P3 complex
PD217887	32	317	RNA-directed RNA Polymerase PB2 transferase subunit

The length of protein sequence in both H5N1 and H1N1 is 759 and as per the result obtained from alignment of both sequences, the identity is 97.5% and similarity is 99.1%.

EMBOSS Align Results

Length: 759
Identity: 740/759 (97.5%)
Similarity: 752/759 (99.1%)
Gaps: 0/759 (0.0%)
Score: 3782.0

The result of SOPMA shows that, there are 284 alpha helices, 176 extended strands, 66 beta turns and 233 random coils are present in its secondary structure. The secondary structure of segment 1 of H5N1 and H1N1 is almost same with very minor changes in its extended strand and random coil structure (**Table 4**).

Table 4: Secondary Structure of Segment 1 Protein of H5N1 and H1N1.

Protein Structure	H5N1	H1N1
Alpha helix	37.42%	37.42%
3 10 helix	0.00%	0.00%
Pi helix	0.00%	0.00%
Beta bridge	0.00%	0.00%
Extended strand	23.19%	23.45%
Beta turn	8.70%	8.70%
Bend region	0.00%	0.00%
Random coil	30.70%	30.43%
Ambiguous states	0.00%	0.00%
Other states	0.00%	0.00%

A recent study has revealed that polymerase B2 component of the 1918 flu virus (H1N1) having a mutation K627E in H5N1 is located inside the virus capsid [13.]. Other research has indicated that this mutation strongly influences the virulence of H5N1. Thus an avian influenza vaccine for humans targeting the polymerase B2 protein inside the capsid is advantageous instead of a vaccine against H or N and it seems reasonable to believe that after the constancy of over more than 80 years, the K627E mutation could now be exploited for developing a vaccine, rather than waiting for new mutations [4]. This also suggests that a peptide-based approach of vaccine development would have the advantage of avoiding the use of dangerous, live, avian influenza virus during mass production [8, 11, 12]. Consequently, a publicly available database at the National Center for Biotechnology Information (NCBI) website and the SYFPEITHI online computer algorithm were used to generate a hypothesis about a peptide based vaccine targeted at the K627E mutation in PB2 of the avian influenza virus [10]. Further, the peptide sequence, DTVQIIKLL present in the PB2 protein of the H5N1 virus would be expected to bind to HLA-A26 restricted immune system cell surface receptors. Hence, the bound peptide might be capable of stimulating protection from cytotoxic T lymphocytes.

Acknowledgements

The authors are thankful to Prof. Dr. M.C. Trivedi, Principal, Government Postgraduate College Rishikesh, Dehradun, Uttrakhand, India for providing the possible necessary facilities to carryout this work.

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