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Hemagglutinin protein of Asian strains of human influenza virus A H1N1 binds to sialic acid - a major component of human airway receptors

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ABSTRACT. Hemagglutinin (HA) protein plays an important role in binding the influenza virus to infected cells and therefore mediates infection. Deposited HA sequences of 86 Asian strains of influenza A (H1N1) viruses during the first outbreak were obtained from the NCBI database and compared. Interaction of the HA protein of influenza A (H1N1) virus with the human sialic acid receptor was also studied using bioinformatics. Overall, not more than three single-point amino acid variants/changes were observed in the HA protein region of influenza A (H1N1) virus from Asian countries when a selected group sequence comparison was made. The bioinformatics

study showed that the HA protein of influenza A (H1N1) binds to the sialic acid receptor in human airway receptors, possibly key to airborne infection in humans.

Key words: H1N1; Molecular simulation; Receptor; Sialic acid

INTRODUCTION

The A (H1N1) pandemic, also commonly referred to as "swine flu", is a global outbreak of a new strain of influenza A virus subtype H1N1, identified in April 2009. Within 5 months after its emergence, infection cases of more than 290,000 with associated deaths of 3486 were reported worldwide, and this number actually understated the real number of cases (Swedish et al., 2010). In Malaysia, as of January 30, 2010, 12,389 infection and 77 death cases have been reported. The flu began in Mexico and Southern California (CDC, 2009). The virus spreads from coughs and sneezes or by touching contaminated surfaces and then touching the nose and mouth (Brankston et al., 2007). The illness manifestations are similar to those of seasonal flu and include fever, cough, sore throat, runny nose, body aches, chills and fatigue, and diarrhea and vomiting have also been reported in some cases. People infected with this virus may be contagious from one day before they develop symptoms to up to 7 days after they get sick (Carrat et al., 2008). Antiviral drugs that are recommended for use against swine influenza A (H1N1) virus are oseltamivir (Tamiflu) and zanamivir (Relenza). As for the vaccine, the influenza A (H1N1) 2009 monovalent vaccine is used and as of January 2, 2010, an estimated 20.3% of the U.S. population (61 million persons) had been vaccinated (CDC, 2010). Malaysia also received 46,000 and 78,000 doses of vaccine in November 2009 and January 2010, respectively. Actions such as containments, quarantines, airport health screenings, school closures, etc., have been undertaken to prevent the spread of the virus and to contain the infection.

The virus isolated from patients in the United States was found to be made up of genetic elements from four different flu viruses - North American Mexican influenza, North American avian influenza, human influenza, and swine influenza virus typically found in Asia and Europe (Novel Swine-Origin Influenza A (H1N1) Virus Investigation Team, 2009). This new strain appears to be a result of reassortment of all four different strains of subtype H1N1. Full-length viral genome sequences from around the world have been published and made available in public-access databases. Influenza A is a single-stranded RNA virus with eight different segments. Six segments of the swine influenza A (H1N1) virus are related to swine viruses from North America and the other two [neuraminidase (NA) and matrix protein (M)] from swine viruses isolated in Europe/Asia (Trifonov et al., 2009a). The segments coding for the polymerase complex (PB1, PB2, PA, PB1-F2), HA, nuclear protein (NP), and nonstructural proteins (NS1, NS2) show high similarity with the swine H1N2 influenza A viruses isolated in North America in the late 1990s. However, the segments coding for the NA and the M1, M2 are distantly related to European swine influenza A virus strain from 1992, with 94.4% similarity at the nucleotide level (Trifonov et al., 2009b). Experts are concerned that the new influenza A (H1N1) virus could mutate over the coming months, noting that the unstable virus could mix and swap genetic material when exposed to other viruses, especially seasonal flu and H5N1 (bird flu) viruses (Novel

Swine-Origin Influenza A (H1N1) Virus Investigation Team, 2009). Fortunately, until July 2009, the swine flu virus has not mutated to become more dangerous (Turner et al., 2009). However, close monitoring of the viral changes is being undertaken as the virus continues to spread extensively around the globe.

In general, HA is an important property of influenza viruses, whereby it is responsible for initial infection in humans through airway receptor attachment. It binds influenza viruses to SA-containing receptors of infected cells and allows the entry of the viral genome into the infected cells by causing the fusion of host endosomal membrane with the viral membrane (White et al., 1997). In this study, we narrowed down the scope and aimed to compare the GenBank-deposited HA protein of new H1N1 viruses from Asian countries to search for possible mutations/variants in the HA protein of this human flu virus during this pandemic. In addition, bioinformatics molecular simulation was also performed to illustrate the binding of the HA protein to sialic acid (SA), a human airway receptor determinant.

MATERIAL AND METHODS

A total of 86 HA protein sequences of human pandemic (H1N1) 2009 viruses from Asian countries during the first outbreak were recruited from Influenza Virus Resource, National Center for Biotechnology Information (NCBI, 2009) (Table 1). An intensive multiple gene sequence alignment was carried out on the full-length HA protein sequence of influenza virus A H1N1. The consensus sequence was obtained and further simulated through homology modeling using SWISS-MODEL server (Guex and Peitsch, 1997; Schwede et al., 2003; Arnold et al., 2006). This model served as the target receptor protein. The SA structure was obtained from a protein databank under PDB accession number IJSO and served as the input ligand. Docking was carried out using ZDOCK (Discovery Studio 2.1).

RESULTS

The multiple gene sequence alignment results indicated that the HA protein was almost fully conserved among all 86 HA sequences of the Asian human pandemic (H1N1) 2009 viruses obtained from the Influenza Virus Resource, NCBI. Not more than three single-point amino acid changes were observed in the HA protein of these influenza A (H1N1) viruses (Table 1). A few deleted amino acid sequences at the 5'- and 3'-end of these 86 HA sequences were not considered mutation points in this study, because they could be due to incomplete sequencing results. Generally, two fragmented or monomer of HA protein models were obtained with the SWISS MODEL, using 1ruyH and 1ru7D as the templates; this was done to maintain the similarity as high as 84.52 and 94.5%, respectively. Both HA models were subjected to docking using Discovery Studio 2.1 with ZRANK, and the top view of the docking results is shown in Figure 1. The SA model, representing the human type receptor, is clearly seen to form a stable complex with the HA protein of the influenza A (H1N1) virus. A further insight analysis demonstrated that o-SA binds to 3 amino acids (GLY357, TRP358 and THR359) of the monomer of the HA protein (Figure 2) and that they are conserved in all 86 samples analyzed.

Table 1. Mutations/variations detected in HA protein of human influenza virus A (H1N1) in Asian countries.

Accession	Country	Year	Mutations/variations
ACU29979	Taiwan	2009/05/19	T14A, S220T
ACU29989	Taiwan	2009/05/22	S220T
ACU29999	Taiwan	2009/05/22	S220T
ACU30009	Taiwan	2009/05/28	K2E, N557G, Q310H
ACU30019	Taiwan	2009/05/30	S220T
ACR38877	China	2009/05/10	K2E, Q310H, K419T
ACR46986	Japan	2009	-
ACR46987	Japan	2009	N387K
ACR46988	Japan	2009/05/17	-
ACR46989	Japan	2009/05/17	-
ACR46990	Japan	2009	-
ACR46991	Japan	2009/05/16	V169I
ACR46992	Japan	2009/05/16	-
ACR46993	Japan	2009/05/16	-
ACR46994	Japan	2009/05/17	-
ACR49236	China	2009/05/17	-
ACR54002	China	2009/05/21	-
ACR54045	China	2009/05/29	S220T
ACT79624	China	2009/06/06	Q310H
ACT79625	China	2009/05/28	A151T, S220T, V428I, E447K
ACU13122	China	2009/06/17	L49I
ACU13129	China	2009/06/29	S220T, D219E, K412E
ACU27041	China	2009/06/13	-
BAH95823	Japan	2009/06/03	Y323H
BAH95824	Japan	2009/05/21	-
ACQ99682	Thailand	2009/05	I421M
ACR01014	Thailand	2009	I421M
ACR78154	Philippines	2009/05/23	S220T
ACR78158	Philippines	2009/05/20	L49I
ACR78164	Philippines	2009/05/25	S220T
ACR78165	Philippines	2009/05/25	H313Y
ACR83538	China	2009/05/29	S220T, Q352L
ACS27780	China	2009/05/24	S220T, V428I
ACS27787	China	2009/05/31	S220T
ACS92569	Japan	2009/06/07	S100P, A214T, V338I
ACS92579	Japan	2009/06/07	S100P, S220T, V338I
ACS92589	Japan	2009/06/02	-
ACS92600	Japan	2009/06/16	S202N
ACT66162	Singapore	2009/06/30	K2E, Q121H
ACT67255	Japan	2009	S220T
ACU56931	Kazakhstan	2009	S220T, D219E
ACT79133	Japan	2009/06/29	S220T, T249K
ACQ84451	South Korea	2009/05/02	-
ACR54047	China	2009/05/18	V520I
ACR54964	China	2009/05/23	S220T, T408I
ACR54974	China	2009/05/23	S220T, V428I, V520I
ACR54984	China	2009/05/22	S220T
ACR54994	China	2009/05/15	-
ACR55004	China	2009/05/20	-
ACR67244	China	2009/05/27	S220T
ACR67254	China	2009/05/23	A278V
ACT10316	Hong Kong	2009/06/11	S220T, D219E, P314S
ACT21941	China	2009/06	L49I, Q240R
ACT22031	Japan	2009/06/11	S220T
ACT22032	Japan	2009/06/23	S220T
ACT22033	Japan	2009/06/09	S220T
ACT22034	Japan	2009/06/18	S220T
ACT22035	Japan	2009/06/13	S100P, A214T, V338I
ACT22036	Japan	2009	V169I
ACT22037	Japan	2009/06/11	S220T, D219E
ACT22038	Japan	2009/06/16	S202N
ACT21572	Israel	2009/04	L49I, D291E

Continued on next page

Table 1. Continued.

Accession	Country	Year	Mutations/variations
ACT21579	Israel	2009/04	L49I, D291E
ACT21584	Israel	2009/04	-
ACR09395	Japan	2009/05/08	-
ACR09396	Japan	2009/05/08	-
ACR32986	China	2009/05/10	L49I
ACR15758	Israel	2009/04	R450K
ACR18920	Hong Kong	2009/04/30	-
ACR23302	Thailand	2009/05/06	I421M
ACR32998	China	2009/05/16	-
ACS34705	China	2009/06/13	-
ACS34966	Japan	2009/05/18	-
ACS34967	Japan	2009/05/21	L329S, N387K
ACS34968	Japan	2009/05/22	T99S, T258I
ACS36632	Japan	2009/05/21	N387K
ACS36645	China	2009/05/18	A158T, V520I, N472S
ACS45017	China	2009/06/18	R222K, I527V
ACS54259	Japan	2009/06/07	S100P, A214T, V338I
ACS54260	Japan	2009/05/19	-
ACS54261	Japan	2009/06/06	S220T
ACS54262	Japan	2009/06/02	K2E, V149I, Q310H
ACS54263	Japan	2009/06/09	S220T
ACS54301	China	2009/05/28	S220T
ACT10838	China	2009/05/20	-
ACS68822	China	2009/05/31	E117G, S220T, V428I

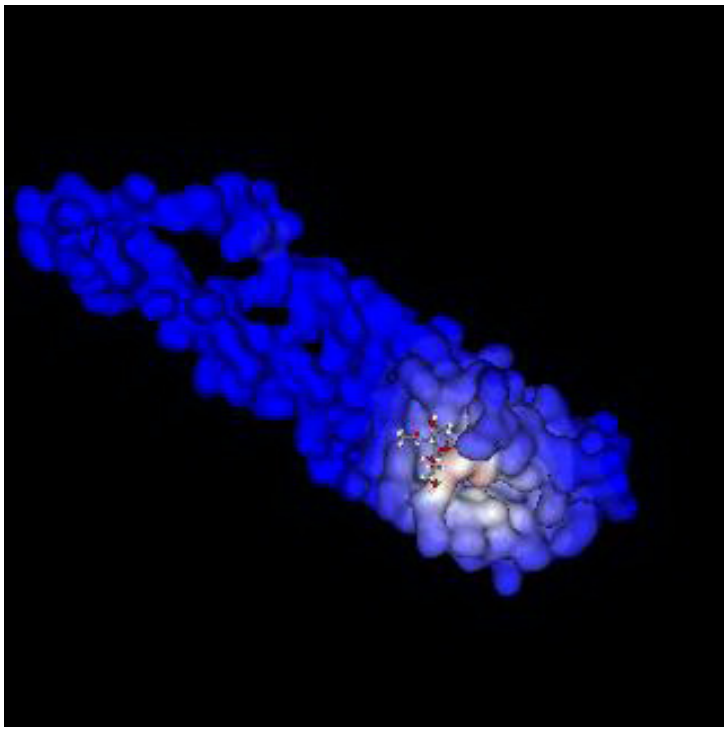

Figure 1. Interaction between sialic acid (SA) and the monomer HA protein of influenza virus A (H1N1). This is the top view of the docking results. The HA protein model is shown in CPK format (blue). The SA is shown in ball and stick format.

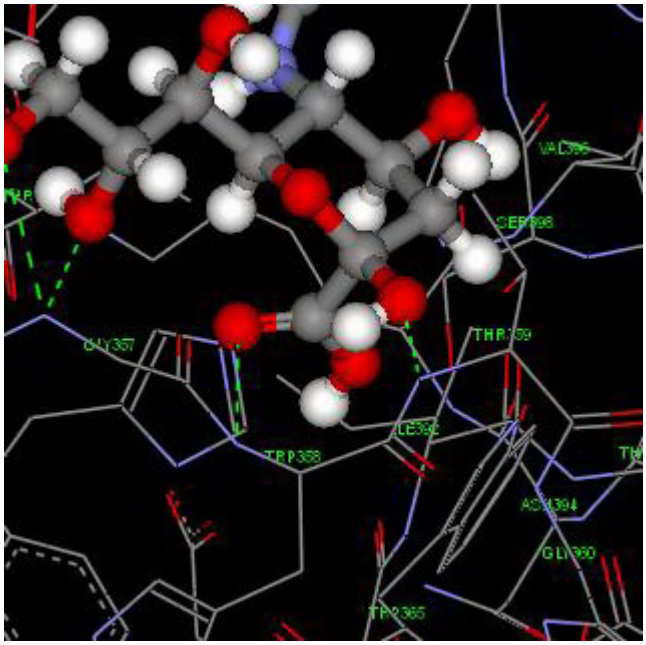

Figure 2. Intermolecule interaction between the HA protein of H1N1 and o-SA. The model shows strong hydrogen bonding between the ligand and the HA protein at positions GLY357, TRP358 and THR359.

DISCUSSION

Influenza pandemics occur when an influenza virus with an HA emerges in the human population and is efficiently transmitted from human to human (Garten et al., 2009). Sixteen subtypes of HA have been identified and the influenza A (H1N1) is the influenza A virus with the HA subtype H1. HA is located outside the virus membrane. In our study, the majority of HA proteins of the 86 influenza A (H1N1) viruses isolated from Asian patients indicated stability in their sequence during this pandemic by only having a few amino acid changes. There was no major mutation, which can possibly change the affinity of HA to human receptors and consequently alter the virulence of influenza A (H1N1). Hence, if the pandemic gets worse in the second wave, it is most likely not only because of HA mutation, that is, NA is the other protein that we need to study. Notably, resistance to oseltamivir has been reported in many countries, and it can occur by only single amino acid substitution in the enzyme NA (Ward et al., 2005). Likewise, close monitoring of viruses by a WHO network of laboratories also shows that viruses from all outbreaks remain virtually identical. No signs of virus mutation to a more virulent or lethal form have been detected (Turner et al., 2009).

In general, a complete HA protein segment is a homotrimer and shaped like a cylinder. It is approximately 13.5 nm long. Each HA monomer is synthesized as a single polypeptide that is cleaved by proteases into two subunits (HA1 and HA2). HA1 contains the receptor-binding and antigenic domains, whereas the HA2 subunit is responsible for the fusion of the virion with the endosomal membrane in the host cell (Wilson and Cox 1990). The HA1

subunit undergoes a process known as positive Darwinian selection through continuous antigenic mutations that allows the virus to evade the host's humoral immune response (Fitch et al., 1991). The 3 spherical heads of HA bind to receptors containing glycans with terminal SAs on the cell surface in the respiratory tract, where their precise linkage determines species preference (Stevens et al., 2006). Our study demonstrated a perfect binding of HA of influenza A (H1N1) virus to human o-SA via hydrogen bonding (Figures 1 and 2). Amino acid substitutions within the receptor-binding pocket or the "second shell" residues may alter the specificity and affinity of the influenza A virus HA toward certain types of galactosidic linkages, namely SA-α2,3-galactose (SAα2,3Gal) (avian) or SAα2,6Gal (human) linkages (Aytay and Schulze, 1991; Matrosovich et al., 2000). In the human respiratory tract, the SAs most dominantly expressed are those linked with SAα2,6Gal (upper airway) and SAα2,3Gal (alveoli and the terminal bronchiole) (Shinya et al., 2006). The affinity of HA attachment to SA is deemed a crucial component in the species barrier that keeps avian influenza viruses from readily infecting humans (Nicholls et al., 2009). Both avian and human-like SAs are present in pig respiratory epithelium and can be infected with both human and avian influenza viruses. Thus, pig becomes the "mixing vessel" where reassortment of avian and human viruses can take place, potentially leading to the emergence of novel influenza strains and causing pandemics (Ito et al., 1998). A very good example of this situation is the currently occurring swine influenza A (H1N1) pandemic, whereby the speed of transmission and fatality number of the infection are considerably alarming. Overall, the HA protein of influenza A (H1N1) virus can bind to human SA and lead to infection. No major mutation was observed in the HA sequence among viruses isolated from Asian patients during this outbreak.

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