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Drug design for Influenza A virus subtype H1N1

Chien-Yu Chen [a], Hung-Jin Huang [a], Fuu-Jen Tsai [b], Calvin Yu-Chian Chen [a,c,*]

[a] Laboratory of Computational and System Biology, School of Chinese Medicine, China Medical University, Taichung, 40402, Taiwan
[b] Department of Medical Genetics, Pediatrics and Medical Research, China Medical University Hospital and College of Chinese Medicine, Taichung, 40402, Taiwan
[c] Department of Bioinformatics, Asia University, Taichung, 41354, Taiwan

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ABSTRACT

An outbreak of influenza A virus subtype H1N1, also known as swine flu, in Mexico was occurred in April 2009. To design drugs for treating this epidemic is urgency. In this study, we employed the new sequences (2009) to build the N1 simulation structure by homology modeling, which has been checked for high reliability by Verify Score and Ramachandran plot. The latest H1 homology model was employed from Chen's report. 365,602 compounds from NCI database have been screened by docking study of H1 and N1, respectively. And then, nine candidates were screened and suggested as potent dual target candidates from the docking studies. In our investigation, drug resistance was found by our molecular simulation in the new N1 modeling structure to oseltamivir. However, the mechanism is still not clear; further clinical investigations are urgently required.

1. Introduction

Since March 2009, an outbreak of H1N1 influenza in Mexico has led to hundreds of confirmed cases and a number of deaths. On April 28, the new strain was suspected to infect more than 2500 individuals worldwide and 152 attributed deaths. The U.S. Centers for Disease Control and Prevention warned that the outbreak could be pandemic. On April 27, 2009, the World Health Organization raised their alertness level from 3 to 4 worldwide in response to sustain human-to-human transfer of the virus, and the situation was raised to level 5 on April 29. Moreover, on June 11, 2009, the WHO declared an H1N1 pandemic, moving the alert level to phase 6, marking the first global pandemic since 1968. Hence, there is an urgent need to find the resolution for this international problem. Unfortunately, H1N1 virus was reported that it has gained drug resistant for oseltamivir (Collins *et al.*, 2008; Hauge *et al.*, 2009; Moscona, 2009). Hence, a new drug is required against this epidemic.

The membranes of influenza virus contain haemagglutinin (HA) and neuraminidase (NA), they both are glycoproteins. Haemagglutinin has 16 subtypes (H1, H2, H3, . . ., H16) and neuraminidase (N1, N2, N3, . . ., N9) has 9 subtypes. They assort the type of influenza A viruses (Mukhtar *et al.*, 2007; Shirvan *et al.*, 2007). Cell-surface sialic acid receptor to bind to initiate virus infection was mediated by HA, and sialic acid was removed from virus by NA. By the above two steps, cellular glycoproteins improve virus releasing and the spread of infection to new cells, respectively (Raymond and Leach, 2007; Takabatake *et al.*, 2007). By blocking haemagglutinin or neuraminidase could prevent virus from invading into host cells (Russell *et al.*, 2006; Shimbo *et al.*, 2007). Both zanamivir (Relenza) and oseltamivir (Tamiflu) are neuraminidase inhibitors (Collins *et al.*, 2008; Ho *et al.*, 2007). Influenza A virus subtype H1N1 is the most common cause of influenza in humans (Palese, 2004). Some strains of H1N1 are human endemic; such as the pandemic flu in 1918, 50–100 million people were killed worldwide (Kash *et al.*, 2006; Kobasa *et al.*, 2007). Less virulent H1N1 strains which roughly caused half of flu infections in 2006 has still existed (Cheung *et al.*, 2002; Kash *et al.*, 2006; Kobasa *et al.*, 2007; Palese, 2004); other strains of H1N1 in swine and fowls are endemic. In the past few years, many reports indicated that virtual screening techniques were feasible (Chen and Chen, 2007; Chen, 2008a,b,c; Chen, 2009a,b,c; Chen *et al.*, 2008, 2009a, b). The experimental procedure flow chart was revealed in Fig. 1. In this

* Corresponding author. Current address: Department of Computational and Systems Biology, Massachusetts Institute of Technology, Cambridge, MA 02139, USA.
E-mail address: ycc@mail.cmu.edu.tw (C.Y.-C. Chen).

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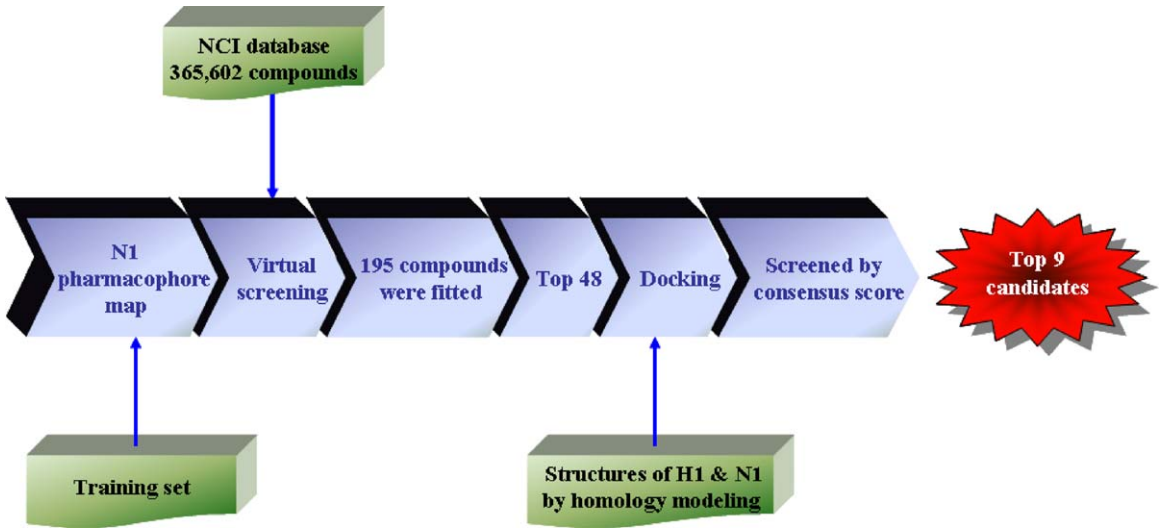

Fig. 1. The flow chart of overall experimental procedures in this study.

study, we have built the latest N1 structure model by homology modeling. In the other hand, the latest H1 homology model was employed from Chen's report (Chen *et al.*, 2009a, b). 365,602 compounds from NCI database have been screened by docking study of H1 and N1, respectively. We aimed at figuring out potent candidates for N1 and H1 for the 2009 outbreak of influenza A H1N1.

2. Materials and methods

2.1. Sequence alignment and homology modeling

All programs in this study were performed by Discovery Studio 2.0 (Accelrys, San Diego, CA, USA). The new sequences (2009) of H1 and N1 were downloaded from NCBI influenza virus sequence database. The templates of H1 and N1 were downloaded from protein data bank (PDB). Their structures had been released in 2004 and 2006, respectively (PDB ID: 1RD8 and 2HU0). The multiple sequence alignment method was based on the CLUSTAL W program and progressive pairwise alignment algorithm (Thompson *et al.*, 1994). The alignment scoring matrix was set in BLOSM by default. 1RD8 and 2HU0 were applied to build the latest structure of the H1 and N1 sequence, respectively.

2.2. NCI database screening

NCI database, which contented 365,602 compounds, was provided by National Center for High-performance Computing. The catalyst compare/fit algorithm was employed to screen the compounds from NCI database, and then, the docking protocol of LigandFit was used to rank the compounds by scoring functions.

2.3. Docking study

All of the compounds were built and energy minimized under MM2 force field by ChemOffice 2005. The LigandFit program performed the docking simulation at the binding site by Discovery Studio 2.0. During the docking procedure, ligands were flexible whereas the receptor was fixed. The ligand flexibility was carried out by In Situ Ligand Minimization based on CHARMm force field. Docking score (DS) was employed to score the docking results. Candidate ligand poses are evaluated and prioritized according to the DockScore function. There are two types of DockScore. One is based on a force field approximation, the other on the Piecewise Linear Potential function (PLP)

$$\text{DockScore (force field)} = -\left(\frac{\text{ligand}}{\text{receptor interaction energy}}\right) - \text{ligand internal energy} \quad (1)$$

$$\text{DockScore (PLP)} = -(\text{PLP potential}) \quad (2)$$

As shown in Eq. (1), there are two energy terms in the force field version of DockScore, internal energy of the ligand and the interaction energy of the ligand with the receptor. The interaction energy is taken as the sum of the van der Waals energy and electrostatic energy. The computation of the interaction energy can be quite time consuming. To reduce the time needed for this calculation, a grid-based estimation of the ligand/receptor interaction energy is employed. Piecewise Linear Potential is a fast, simple, docking function that has been shown to correlate well with protein–ligand binding affinities. PLP scores are measured in arbitrary units, with negative PLP scores reported in order to make them suitable for subsequent use in consensus score calculations. Higher PLP scores indicate stronger receptor–ligand binding (larger pK_i values). Additionally, PMF was computed by summing pairwise interaction terms

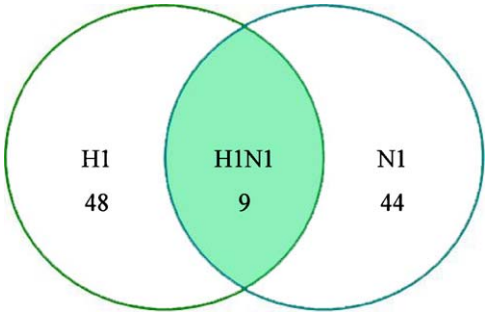

Fig. 2. The screening results of H1 and N1 by docking study. There are 48 and 44 compounds listed in H1 and N1 docking results, respectively. There are 9 compounds overlapped in the set-theoretic intersection.