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Review

In silico approach towards H5N1 virus protein and transcriptomics-based medication

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H5N1 influenza A virus is a serious threat to human population. With a considerable mortality rate, strategies for coping with the infection are being developed. Our research group and some others investigated the potential therapeutic and preventive measures for tackling H5N1 infections. Protein-based and transcriptomics analyses are getting more important in this field. The trends towards the integration of both protein-based and transcriptomics for H5N1 analysis are indeed feasible.

Key words: H5N1, protein-based, transcriptomics, siRNA, hemagglutinin (HA), matrix1 (M1), non-structural 1 (NS1), neuraminidase (NA), and matrix2 (M2).

INTRODUCTION

H5N1 influenza A virus, which is a highly pathogenic avian influenza (HPAI), has inflicted massive mortality in humans and poultry (Peiris et al., 2007). The whole Asia has been incurred by this virus. The H1N1 subtype (Spanish flu) caused much causality in 1918, with another occurrence again in Mexico (Swine flu) in 2009, causing many casualties as well (Asmara et al., 2005). The fatality of HPAI H5N1 threat must be further evaluated due to its economic impact. The complex nature of H5N1 infection has encouraged researchers to study its molecular mechanisms in order to develop the remedy (Peiris et al., 2007). The antigenic drift in H5N1 that occurred due to its evolutionary course has enabled it to be transmitted from human to human, posing a terrible pandemic threat (Peiris et al., 2007).

Out of the three strains of influenza viruses, A, B and C, type A is the most deadly of all, being responsible for epidemics that have been occurring every 10 to 40 years (Baigent and John, 2003). As a member of Orthomyxoviridae family, influenza virus A has eight segments of single stranded RNA: hemagglutinin (HA), neuraminidase (NA), polymerase basic 2 (PB2), polymerase basic 1 (PB1), polymerase acidic (PA), non-structural (NS), nucleoprotein (NP), and matrix (M). Till date, there are 16 detected types of HA and nine types of NA (Cox et al., 2005).

The most important phase in the H5N1 infection is the viral attachment to sialic acid receptors in the surface of the host cell. The HA protein of avian influenza virus could only identify bird's [Neu5Ac (α2-3) Gal] receptors, and not that of humans [Neu5Ac (α2-6) Gal]. This receptor difference should disable the avian influenza virus from infect humans, but the possible antigenic drift/shift in HA has opened this possibility of global pandemic threat. It is suggested that mutation in other genes (that is, NA, PB1, PB2, NS, and M) could also boost the pathogenicity of H5N1 virus (Asmara et al., 2005). Low pathogenic form of mild avian influenza (LPAI) is also available besides HPAI; but the morbidity rate in HPAI could reach 100% (Asmara et al., 2005).

Our research group has conducted research on HA, M1, NS1, NA, and M2 protein mutations of H5N1 (Tambunan et al., 2012, 2010). However, we are aware of the protein-based and transcriptomic based research in other laboratories. The objective of this review was to compare both type of researches and determine the feasibility of transcriptomics and protein-based approach in coping

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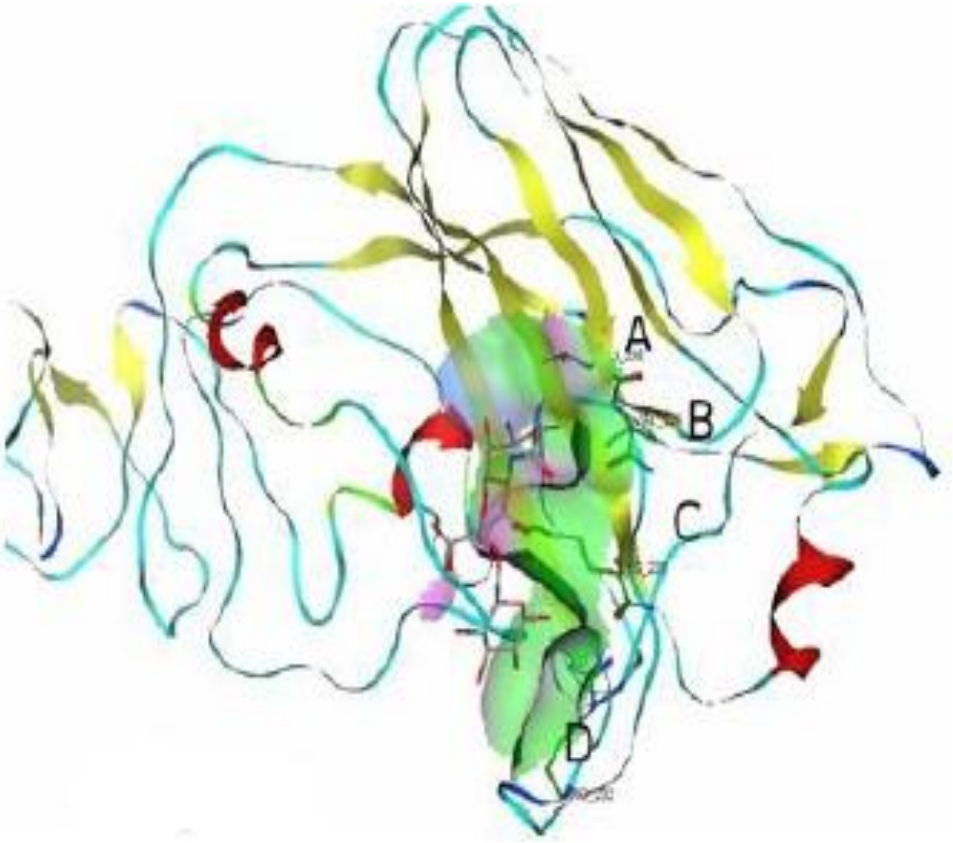

Figure 1. Docking visualization of HA with sialic acid. A, Ile_225; B, Val_226; C, Lys_228; D, Arg_232. Figure taken from Usman et al. (2010), Figure 4.

with H5N1 infections.

PROTEIN-BASED DISCOVERIES FOR H5N1 STUDIES

Our method

We already conducted research on H5N1 computation that focused primarily on mutation studies. Our findings can be easily applied to a drug development study.

The pathogenicity of influenza virus could be influenced by mutations with either antigenic shift or antigenic drift (Tambunan et al., 2010). HA, NA, and M2 are known to have important roles in the infection process of avian influenza virus. As usual, multiple alignment and phylogenetic tree construction were used for the *in silico* analyses. Both the cleavage and active sites underwent mutations of HA; with the active site also undergoing NA and M2 mutations. Hydrophilic to hydrophobic shift of the amino acid character influences the pathogenicity of the virus. Pro-P prediction was used for the mutation analysis result as well as 3D structure prediction, molecular docking simulation and molecular dynamics simulation. R-X-K/R-R pattern was obtained for H5N1 Indonesia and Hongkong due to the mutation analysis on HA cleavage site. Furin cleavage of HPAI H5N1's HA was shown by the pattern of Pro-P prediction. The strong binding of HA and NA H5N1 Indonesia towards human sialic acid receptor was analyzed by using molecular docking and molecular dynamics. However, amantadine and rimantadine are resisted by the activity M2 protein of H5N1 virus. Figure 1 shows the visualization of HA with sialic acid. Overall, there is a strong relation between the pathogenicity of H5N1 in Indonesia and the mutation of HA, NA and M2 based upon their binding with sialic acid receptors (Tambunan et al., 2010).

The latest research of Tambunan et al. (2012) was done through the observation of NS1 and M1 mutations in their entire region and the cleavage site of HA protein. With established methodology in Tambunan et al. (2010), the observation was followed by prediction of the pro-P (furin) HA specific cleavage, secondary structure, mutation (exposed/buried) prediction, epitope prediction, and 3-D structures prediction. RXK/RR conserved pattern of the mutation of HA cleavage site was found in Hong Kong H5N1, while the sequences of subtype H1N1, H1N2, and H3N2 lacked it. The cleavage of the HA of H5N1 is possible based on the detected pattern by the furin predictor. The position 53 of the mutation of NS1 control sequences was found in A/Indonesia/5/2005(H5N1), A/Indonesia/CDC1032/2007(H5N1), A/HongKong/156/97

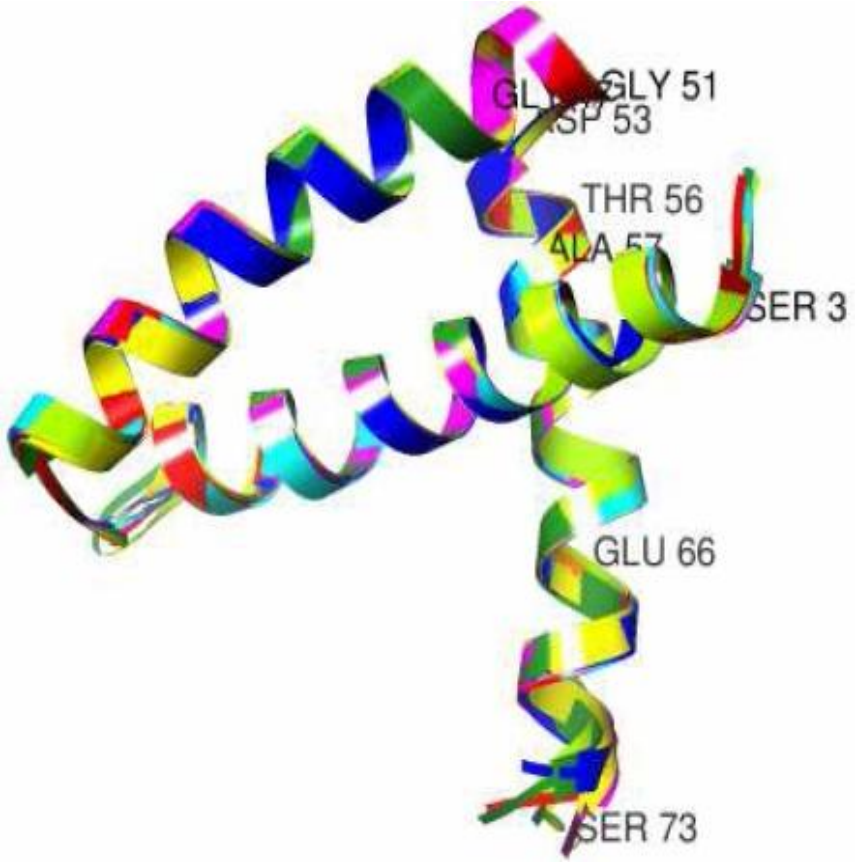

Figure 2. Superimposed RNA binding domain NS1. Figure taken from Usman et al. (2012), Figure 1.

(H5N1), A/BrevigMission/1/18(H1N1), A/Mexico /InDRE4487/2009 (H1N1) with the subtypes H1N1, H1N2, and H3N2. However, these subspecies have no identical specific control mutation on the M1. There are 248 changed positions in H1N1 and H3N2. The control sequences NS1 and M1 of H1N1, H1N2 and H3N2 subtypes have similar inhibitory concentration at 50% (IC_{50}) values below 50 nM based upon epitope prediction. However, the epitope recognition did not find any specific mutations. Secondary and tertiary structures of NS1 and M1 proteins were not significantly affected by the region-specific mutations compared with the control sequences (Tambunan et al., 2012). Figure 2 shows the superimposed RNA Binding Domain of NS1.

Discovery of other laboratories

The nuclear magnetic resonance (NMR) spectroscopy has already successfully determined the 3D structures of the M2 proton channels for influenza A (Schnell et al., 2008) and B (Wang et al., 2009) viruses. Synchronization of histidine protonation and opening of the channel gate have been emphasized by Pielak and Chou (2010). Moreover, destabilization of M2 helix-helix assembly by drug-resistant mutants could impair drug binding (Pielak et al., 2009). Allosteric mechanism of M2 NMR structure was supported by it being energetic, the channel-gating dynamic process, pK(a) shift, its impact on the channel and its consistency with previous functional studies (Du et al., 2009).

Mutations of D44N, D44A and N44D on position 44, and mutations on positions 27 to 38 in M2 have been studied thoroughly for their functional features (Huang et al., 2008). On one hand, M2 ion-channel could be inhibited by adamantine, amantadine and rimantadine antiviral drugs which start to be ineffective due to mutations (De Clercq, 2006; De Jong et al., 2005). On the other hand, six analog inhibitors for H5N1 virus were proposed by Du et al. (2007). A series of docking studies have singled out nine analogs of AG7088, and zanamivir and oseltamivir have worse binding energy than the compound AG7088 (Gong et al., 2009). The reduction of the high pathogenicity of the virus could be fitted well if some mutations of H5N1 H5 cleavage sequence happened to disrupt the fitting into the furin. The sub-sites P(1), P(4) and P(6) from those findings could be focused on developing H5N1 drugs. Wang et al. (2007) conducted computational efforts to analyze BAE46950 sequence (homology model

of H5N1-NA), with 21 sequences found from the database of the 108 H5N1 NAs with over 95% sequence similarity with BAE46950.

Moreover, the subtle mutated variations of the H5N1-NA that change its complement interaction were found by Wei et al. (2006). The high resistance against existing NA inhibitors could be caused by those mutations. The formation of stronger inhibition power than oseltamivir was possible by three lead compound derivatives of Wang et al. (2010). Henceforth, development of new and more powerful drugs for treating influenza could be pursued by the utilization of those candidates. These advancements in the field of protein-based computation could possibly lead to the development of new therapeutic and preventive agents.

TRANSCRIPTOMICS STUDIES OF H5N1 VIRUS

According to the study of Koparde and Singh (2011), the silencing of the pathogenic gene products associated with viral infections could be utilized by RNA interference (RNAi) mediated by microRNA (miRNA). Koparde and Singh (2011) have reviewed the role of miRNA in its pathophysiology and the computational prediction of miRNA as antiviral therapeutics. The highly variable nature of influenza antigenic proteins could eventually break down the efficacy of the conventional influenza drugs. The fund-saving nature of *in silico* prediction of sequence specific therapeutics would be helpful in determining the miRNA target prediction platforms, which are very different in their specificity and sensitivity in prediction.

Moreover, Koparde and Singh (2010) also propose a bioinformatics pipeline to convey prediction of microRNAs that posed activity against non-structural protein 1 of H5N1 and segment 8 of H1N1 virus. They found several potential sequence specific miRNA therapeutic agents against NS1 protein of H5N1 virus. Those are hsa-miR-138, hsa-miR-525-5p and hsa-miR-124. The possible anti-influenza agents were extracted from the different H1N1 segment 8 genomes that resulted from the predic-tion of miRNAs.

The regulatory processes of host cell, stress related pathways including mitogen-activated protein kinase (MAPK) and mammalian target of rapamycin (mTOR) path ways are predicted to be regulated by the micro RNAs. Those predicted miRNAs are also expected to be involved in pathways related to cancer (Koparde and Singh, 2010).

ElHefnawi et al. (2011) designed optimal small interfering RNA molecules (siRNAs) for targeting all diverse influenza A viral strains. The analysis performed on possible influenza-targeting siRNAs resulted from the rational integration of the highly efficient design in the pipeline.

The siRNA that has the ability to target highly conserved, an accessible and biologically significant region has been selected in this analysis, it only requires minimal dosage and side effects. The design of more than 6000 possible siRNA was devised. The elimination of those with off-targets in the human genome and those with undesirable properties and selection of siRNA tar-geting highly probable single-stranded regions were done as further filtration of siRNAs. The selection conserved and biologically functional short motifs were the final stage of the optimization of the properties of siRNA. The fertility of this methodology was shown by validation of a predicted active (sh114) and a predicted inactive (sh113) (that was filtered out in Stage 8) silencer of the *NS1* gene that eventually showed significant inhibition of the *NS1* gene for sh114, with negligible decrease for sh113 which failed target accessibility (ElHefnawi et al., 2011). These findings in the field of transcriptomics are promising for utilization as drug or vaccines.

FUTURE PROSPECTS: INTEGRATION OF PROTEIN-BASED AND TRANSCRIPTOMICS

Advanced researches of protein informatics have already been intersected with the realm of transcriptomics. Some research groups have shown that the protein repertoire of the organism is indeed exposing its transcriptomics properties. Parikesit et al. (2011) have already worked on domain co-occurrences and Moore et al. (2012), on domain loss/gain. Parikesit et al. (2011) found that transcription factor domains are having tendency to avoid each other on multicellular organism, while co-occurrence happens on the simple/basal organism. Moore et al. (2012) also found that optomotor domain, which co-occurs with members of the T-box family, an ancient transcriptional regulator, is emerging in *Drosophila*. This protein-based discovery has exposed the potential transcriptomic expression of the living entities. They have eventually annotated an important protein repertoire with roles in transcription factors, which are the cardinal requirements for transcriptomics expression. The research on transcription factor domain repertoire is eventually leading to more emphasis on its interrelationship with transcriptomic that would be useful on virology informatics. It could be inferred that extensive protein-based studies will lead to the deeper understanding of transcriptomics.

There are some research groups who have already realized the potential of transcriptomics and proteomics integration. Com et al. (2011) successfully provided comprehensive evaluation on integration of transcriptomics and proteomics on gentamicin nephrotoxicity in rats. Meanwhile, Piruzian et al. (2010) have proposed a pipeline for network analysis towards integration of transcriptomics and proteomics in psoriasis. Their researches are the important motivation for the creation of a proteomic and transcriptomic therapeutic library.

Within our future work, we plan to apply the integration of protein-based and transcriptomics data for developing a comprehensive H5N1 drug design library. We will work more on transcriptomics research as well, since we already possess the necessary skill for it. The research of Hofacker et al. (1998, 1999, 2002) have shown that transcript-

tomic expression of viral genome could be annotated. Moreover, having a more complete viral protein annotation, using gene prediction package would be a feasible option as well (Parikesit et al., 2011). It would be interesting to exploit more viral bioinformatics research using both approaches for producing more biological conclusion for drugs development.

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