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Review

Multi-Omics Studies towards Novel Modulators of Influenza A Virus-Host Interaction

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Abstract: Human influenza A viruses (IAVs) cause global pandemics and epidemics. These viruses evolve rapidly, making current treatment options ineffective. To identify novel modulators of IAV-host interactions, we re-analyzed our recent transcriptomics, metabolomics, proteomics, phosphoproteomics, and genomics/virtual ligand screening data. We identified 713 potential modulators targeting 200 cellular and two viral proteins. Anti-influenza activity for 48 of them has been reported previously, whereas the antiviral efficacy of the remaining 665 is unknown. Studying anti-influenza efficacy, immuno-modulating properties and potential resistance of these compounds or their combinations may lead to the discovery of novel modulators of IAV-host interactions, which might be more effective than the currently available anti-influenza therapeutics.

Keywords: influenza virus; antiviral agent; proteomics; phosphoproteomics; metabolomics; transcriptomics; genomics; virtual ligand screening

1. Introduction

Influenza A viruses (IAVs) mutate extremely fast and this rapid evolution enables emerging viruses to evade the prevailing immunity in the human population and to cause global epidemics and pandemics [1]. Several antiviral drugs have been developed in order to combat influenza outbreaks [2]. In particular, amantadine, rimantadine, oseltamivir, zanamivir, laninamivir, and peramivir are approved for treatment of IAV infections. However, almost all IAV strains are resistant to amantadine and rimantadine, due to mutations in viral proton-channel M2 (www.influenzareport.com/ir/drugs/amanta.htm). Oseltamivir is widely used for treating seasonal IAV infections; however, oseltamivir-resistant IAV strains emerge and reduce the efficacy of the treatment due to mutations in viral neuraminidase (NA) [3]. The critical question remains: what will be the next generation of influenza antivirals?

Dozens of novel antiviral drugs are currently under development [4, 5]. For example, DAS181, JNJ872, ribavirin, verdinexor, 202 CH65, C05, SaliPhe, nucleozin, geldanamycin, 17-AAG, LJ001, SA-19, fattiviracin, TBHQ, 4C, gemcitabine, ASN2, bortezamib, carfilzomib, C75, 25HC, SNS-032, MK2206, as well as many other IAV- and host cell-directed agents are currently under pre-clinical or clinical investigations [4-8]. Some of these, or other antiviral agents under development, could replace

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the conventional anti-influenza therapeutics in the nearest future. However, more information about virus-host interactions is needed in order to improve the treatment options for viral diseases. Nowadays, various omics techniques can successfully be used for retrieving information about virus-host interactions on the proteome, transcriptome, post-translational and metabolome levels. The integration of this data could be utilized for the development and identification of modulators of infection and potential antiviral drugs. This review attempts to summarize the results of combining transcriptomics, proteomics, phosphoproteomics, metabolomics, and genomics data for identifying potential cellular targets in IAV-host infection.

2. Combination of various omics techniques identifies potential novel modulators of IAV-host interaction

Here, we expand the list of potential druggable modulators of IAV-host cell interactions by re-analyzing our recent multi-omics data, including transcriptomics, proteomics, phosphoproteomics, metabolomics, and genomics/virtual chemical screening (Figure 1).

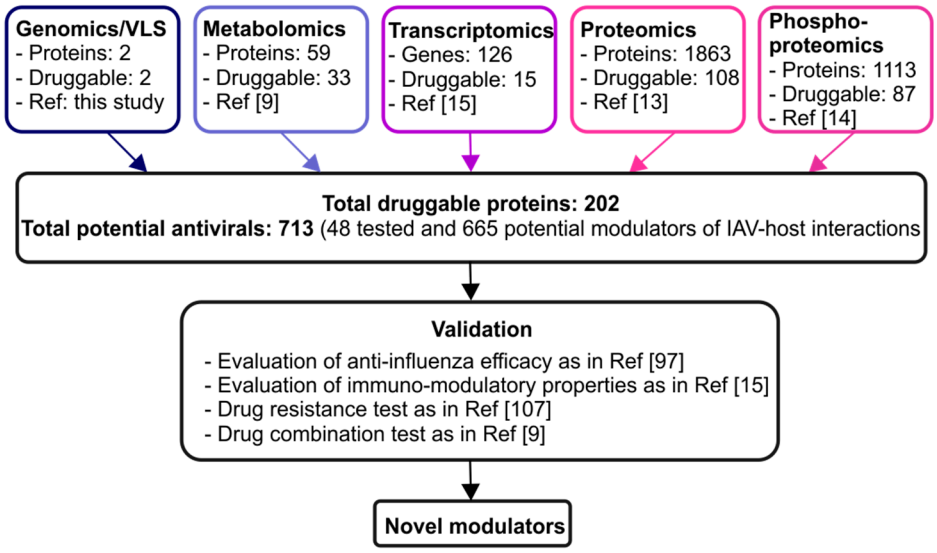

Figure 1. Discovery pipeline for novel modulators of IAV-host interactions.

We recently performed a transcriptomics study using human peripheral blood mononuclear cells (PBMC)-derived macrophages and influenza A/Udorn/1972(H3N2) and A/WSN/1933(H1N1) viruses [9]. We used the Illumina H12 microarray platform, containing more than 47,000 oligonucleotides, representing almost the complete human transcriptome. A total of 126 genes showed a greater than four-fold up- or down-regulation in virus infected cells compared to mock-infected cells at 8 h post infection ($p<0.05$). The most significant canonical pathways specifically associated with virus infections, according to gene set enrichment analysis (GSEA; www.broadinstitute.org/gsea, [10]), were interferon-α, -β and -γ signaling, cytokine signaling, cytokine-cytokine receptor interaction, and cytosolic DNA-sensing pathway. Next, we searched for genes that encode proteins and for which potent chemical/synthetic inhibitors are available, based on the Drug Bank and Drug Gene Interaction Database (http://www.drugbank.ca/; dgidb.genome.wustl.edu/) [11, 12]. In this transcriptomic analyses, we identified 15 proteins, which can be targeted with 53 compounds (Table S1).

We performed quantitative subcellular proteome and secretome studies using human PBMC-derived macrophages and the influenza A/Udorn/1972 strain [13]. We identified 3477 host proteins and reliably quantified 2466 of these proteins using the iTRAQ technique. In total, 1321 proteins were differentially expressed in the intracellular fractions (fold change ≥1.5 or ≤0.67) and 544 in the secretome (fold change ≥3) as a result of infection. We again searched for druggable proteins among

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1865 candidates, using the Drug Bank and Drug Gene Interaction Database [11, 12]. We found 108 proteins, which could be targeted with 346 compounds (Table S1). Interestingly, five of these proteins (tumor necrosis factor; TNF, C-X-C motif chemokine 10; CXCL10, C-C motif chemokine 3; CCL3, Nicotinamide phosphoribosyltransferase; NAMPT, C-C motif chemokine 8; CCL8) were also found among the druggable targets identified in our transcriptomics study.

We also performed phosphoproteomics profiling of human PBMC-derived macrophages infected with A/Udorn/1972 virus at 6 h post infection [14]. Our analysis identified 1675 phosphoproteins in mock and IAV-infected human macrophages. The phosphorylation of 1113 of these proteins was altered upon infection. We searched for proteins, for which chemical/synthetic inhibitors are available using the Drug Bank and Drug Gene Interaction Database [11, 12]. We found 87 phosphoproteins that could be targeted by a total of 382 compounds (Table S1). Among these proteins, there were several cyclin-dependent kinases. Our efficacy studies showed that cyclin-dependent kinase inhibitor SNS-032 efficiently inhibited influenza virus infection *in vitro* and *in vivo* [15]. Interestingly, 38 druggable proteins identified by phosphoproteomics were also identified in our proteomics study (Table S1).

We have also analyzed the metabolic profiles of PBMC-derived macrophages infected with A/Udorn/1972 or A/WSN/1933 strains for 24 h with LC-MS/MS [9]. In particular, we found that the level of tryptophan was decreased and the level of its oxidation product, L-kynurenin, was elevated. This suggested that tryptophan catabolism was activated during IAV infection. Interestingly, in our transcriptomics study, levels of indoleamine 2,3-dioxygenase (IDO), which catalyzes tryptophan oxidation, was also increased 32-fold in IAV infected macrophages in comparison with mock. Similarly, the levels of adenosine, adenine, inosine, inositol monophosphate, and xanthine were altered in IAV infected macrophages, suggesting that purine metabolism was modulated by IAV infection. In line with the metabolomics results, our transcriptomics experiments showed that the expression of NT5C3, PDE4B, PNPT1, GMPR, ENTPD3, and NUDT2 genes (that are involved in purine metabolism) were upregulated in response to infection. We also observed alterations in glutathione, nitrogen, arginine and proline, alanine, asparagine and glutamine, histamine, cysteine and methionine metabolic pathways. The molecules (which are all enzymes) identified in the metabolomics study [9] and which are involved in these pathways, were manually examined in the KEGG database [16]. Several compounds targeting these enzymes were then identified by the Drug Bank database [11]. Altogether, we found 33 potential targets for 102 compounds (Table S1).

We have also performed a genomics/virtual chemical screening (VLS) study using available human influenza A(H3N2) and A(H1N1)pdm09 virus sequences, high-resolution IAV protein structures, and a library of FDA-approved drugs. We first downloaded 4983 whole-genome sequences of influenza A(H1N1)pdm09 and 6385 sequences of influenza A(H3N2) strains from Influenza Virus Resource and Global Initiative on Sharing Avian Influenza Data databases (http://www.ncbi.nlm.nih.gov/genomes/FLU/FLU.html; http://platform.gisaid.org/). We converted the nucleotide sequences to protein sequences. The protein sequences were aligned and similarity rates for each amino acid in the alignments were calculated. We used available X-ray and NMR structures of influenza proteins from the protein databank (http://www.rcsb.org/) to mark highly conserved amino acids (see [9] for details). We identified 25 highly conserved sites on influenza proteins. To identify allosteric and cryptic binding sites for potential influenza antivirals, in addition to known active sites and binding pockets, we applied the Q-MOL molecular surface scanning methodology [7]. Q-MOL allows identification of "hot" spots on the molecular surface of a protein (http://q-mol.com/). Briefly, minimized 3D molecular structures of the individual 20 amino acids were used as probes to systematically scan the molecular surface of a protein target. During the scan, non-bonded interactions were evaluated between an amino acid probe and protein residues in proximity of a probe. This methodology allowed the detection of excess energy stored on the surface of a protein. This excess surface energy makes it possible for small molecules and other ligands to specifically bind to protein targets at a particular spot. We identified several hot spots for each target protein, but focused only on two that overlapped with evolutionary conserved sites on the proton channel M2 and polymerase subunit PA (PDB IDs: 2RLF, 4WSB). We performed VLS for these two

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docking sites using Q-MOL and a library of FDA approved drugs (in total 3655 ligands). After VLS, the ligands were ranked by relative binding energy, sorted, and the seven best hits per target were visually inspected and selected (Table S1).

Altogether, we identified 202 cellular and viral proteins for which 713 inhibitors are available. Interestingly, anti-influenza activity was tested for 48 of these agents (Table S2) [15, 17-91]. In particular, anti-influenza activity was reported for benzbromarone, ambraxol and tannic acid [92-94]. Kinase inhibitors, such as dinaciclib, flavopiridol, SNS-032, and MK2206, and TNF inhibitors, such as etanercept, adalimumab and infliximab, as well as a lipid-lowering simvastatin, and antibacterial vancomycin, rifampicin, and erythromycin were also reported to possess anti-IAV activity [14, 28, 31, 32, 40, 52, 53, 71, 91, 95]. Interestingly, some of the identified inhibitors could be used for treatment of pain and inflammation associated with severe infections [96] (Patent US 20130123345 A1). Our multi-omics studies, however, did not identify some known inhibitors of IAV-host cell interactions, including Mcl1, RNR, Bcl-xL, and Top1 [97-99]. One possibility is that our multi-omics studies provide only a snapshot of interaction between certain influenza A strains and macrophages isolated from different donors and infected at various time points.

Importantly, based on our omics studies we identified 665 small molecules that target the identified genes/proteins. These compounds might represent novel anti-influenza agents. Based on their target protein annotations, they were clustered into signaling/metabolic pathways by searching KEGG and Reactome databases using DAVID functional annotation tools [100]. The representative pathways, the small molecules and their target proteins are visualized in Figure 2 as an "eye diagram" [101]. Especially interesting are the compounds identified to target proteins/genes identified in at least two omics studies. These include NAMPT inhibitors - GMX1777, CANX, ANXA 2, a TRX8 inhibitor - tenecteplase, a FLAP inhibitor - AM103, a NCF2 inhibitor - dextromethorphan, a IGF2R inhibitor - mecasermin, ICAM1 inhibitors - natalizumab and hyaluronic acid, a TPMT inhibitor - olsalazine, and FASN inhibitors - cerulenin and orlistat [102-106]. These small molecules should be first evaluated *in vitro* using antiviral efficacy assays, and then in animal models as described before. The immuno-modulatory effects of these drugs should also be studied, followed by drug resistant tests [15, 107]. In addition, combinations of some of these drugs could be tested, to decrease their toxicity and increase the efficacy of combination treatments [9]. Such follow-up studies would allow identification of safe and effective novel anti-IAV agents. We expect that five to ten novel therapeutics or their combinations could emerge and be used in the future clinical studies.

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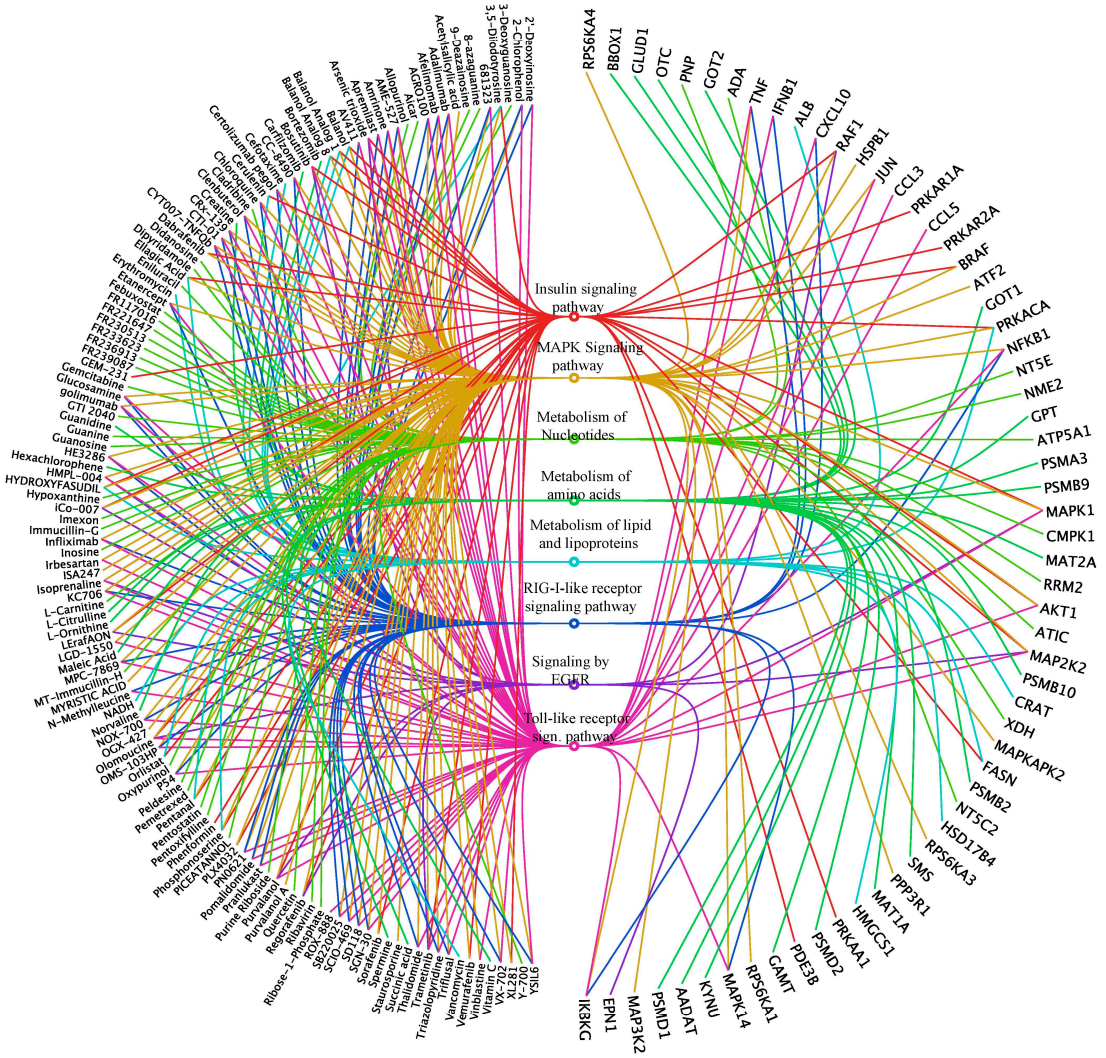

Figure 2. An 'eye diagram' showing representative pathways, proteins and compounds, which can be potentially used for modulation of IAV-host interactions. The signaling/metabolic pathways are represented as various colored circles in the middle of the eye diagram. The colored curved lines originating from the pathways connect corresponding compounds on left to target proteins on right. Only the compounds that have shorter names are included for the sake of clarity.

3. Conclusions

IAVs have evolved mechanisms to disconcert our innate immunity and secure viral replication [7]. IAVs also deceive our adaptive immunity by constantly modifying its proteins [1]. However, our immune system can still limit virus replication and, in the majority of cases, protect us against the development of severe and lethal infections. But there are a substantial number of cases when IAV infection leads to hospitalization and even death of the infected individual (www.who.int). Therefore, more precise understanding of the virus–host interplay might reveal vulnerabilities that can be exploited by direct pharmacological interventions to control and treat IAV infections.

Our recent multi-omics studies identified a total of 202 cellular and viral factors for which 713 targeting agents are available. Importantly, it is known that many of these agents are safe in humans (i.e. data on adverse compound reactions and adverse effects of other treatments in humans are available), because they were originally developed for the treatment of other diseases. Repurposing of these compounds for treating IAV and perhaps other infections could save time and resources in the drug development process. Careful evaluation of these compounds would allow identification of the most potent antiviral agents for further clinical studies. Some of these therapeutics may therefore

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lead to substantial progress in the treatment of IAV infections, and could perhaps be used to control future influenza epidemics and pandemics.

Supplementary Materials: The following are available online at www.mdpi.com/link: Table S1: Genes and proteins implicated in influenza A virus-host interactions and potential modulators of these interactions; Table S2: Forty-eight compounds which have been tested against IAV infection previously.

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Author Contributions: All authors analyzed the data and wrote the manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

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