A Review and Meta-Analysis of Influenza Interactome Studies

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Annually, the influenza virus causes 500,000 deaths worldwide. Influenza-associated mortality and morbidity is especially high among the elderly, children, and patients with chronic diseases. While there are antivirals available against influenza, such as neuraminidase inhibitors and adamantanes, there is growing resistance against these drugs. Thus, there is a need for novel antivirals for resistant influenza strains. Host-directed therapies are a potential strategy for influenza as host processes are conserved and are less prone mutations as compared to virus-directed therapies. A literature search was performed for papers that performed viral–host interaction screens and the Reactome pathway database was used for the bioinformatics analysis. A total of 15 studies were curated and 1717 common interactors were uncovered among all these studies. KEGG analysis, Enrichr analysis, STRING interaction analysis was performed on these interactors. Therefore, we have identified novel host pathways that can be targeted for host-directed therapy against influenza in our review.

Keywords: influenza, host-pathogen interactions, influenza proteins, interactome analysis, bioinformatics

INTRODUCTION

Influenza viruses are negative-sense single-stranded RNA viruses from the Orthomyxoviridae family that cause respiratory diseases (MacLachlan and Dubovi, 2017). Of the 4 influenza virus types, A, B, C, and D, type A is the most prolific as it infects numerous hosts and is the main causative agent of the seasonal and pandemic influenza (Shen et al., 2015). Influenza viruses constantly evolve with antigenic shifts (reassortment of viral segments, resulting in dramatically different viruses) and drifts (small antigenic changes to increase immune evasion). Due to the viral adaptation and reassortment, highly virulent strains may appear and result in local epidemics or global pandemics, such as the 1918 H1N1 Spanish pandemic, 2005 H5N1 Bird flu, and the 2009 H1N1 Swine flu (Shen et al., 2015). Influenza's genome, which is composed of eight segments of symmetrical helixes, encodes ten proteins. Those include the surface glycoproteins haemagglutinin (HA) and neuraminidase (NA), matrix protein (M1), matrix ion channel (M2), Nucleoprotein (NP), PA (polymerase acid subunit),...
polymerase basic subunit 1 (PB1), and polymerase basic subunit 2 (PB2), which form the RNA-dependent RNA polymerase complex, NS1 (non-structural protein 1) and NS2, non-structural protein 2 or nuclear export protein (NEP; Noda, 2012). Segments of some influenza A virus strains may encode a second or third polypeptide in alternative reading frames (McCauley et al., 2012). These functional proteins (Jagger et al., 2012), such as PB1-F2 and PA-X, are known to modulate the host response to the virus (Klemm et al., 2018). The influenza A virus can be further classified based on its HA and NA glycoproteins into different subtypes. There are 18 haemagglutinin and 11 neuraminidase subtypes known today (Tong et al., 2013). Only the H1, H2, H3, N1, and N2 subtypes have caused epidemics in humans (Bouvier and Palese, 2008).

Current Prophylaxis and Treatment of Influenza

Vaccination is available for influenza and is the main form of prevention against influenza. Vaccine efficacy is around 60% if matched to the current circulating strains of the virus, but effectiveness can be as low as 10–20% if there is a mismatch between the vaccine and current strains of the virus (Eisenstein, 2019). The current method of developing influenza vaccines is lengthy, with the Centre for Disease Control (CDC) characterizing around 2,000 influenza viruses. These viruses are monitored for drifts and shifts and compared to viruses included in the current influenza vaccine. This provides an indication of the vaccine’s ability to produce an immune response against current circulating strains of influenza (CDC, 2019). There are three main kinds of vaccines: egg-based vaccines, attenuated vaccines, and recombinant vaccines. However, due to the constantly evolving nature of influenza, there are problems associated with vaccine mismatches (Paules et al., 2018) and poor immunogenicity of vaccines in the elderly (DiazGranados et al., 2014). Moreover, there is a lack of a universal influenza vaccine covering all strains and subtypes of influenza (Krammer et al., 2018).

Hence, there is a need for antiviral therapy for breakthrough infections and for infection with influenza strains not covered by vaccines. M2 ion channel inhibitors (e.g., amantadine and rimantadine) and NA inhibitors (e.g., oseltamivir and zanamivir) are the original two drug classes approved for influenza treatment (Krammer et al., 2018). Resistant virus strains have emerged and have rendered M2 inhibitors ineffective for clinical use while there is also increasing NA inhibitor resistance, such as the H274Y mutation, found in the 2009 H1N1 strain which conferred oseltamivir resistance (Arias et al., 2009; Shen et al., 2015). Recently, baloxavir (cap-dependent endonuclease inhibitor) was approved by the FDA in 2018 and worked by interfering influenza’s ability to multiply via inhibition of viral transcription (Hayden et al., 2018). There are a limited number of compounds under development or in trials (Davidson, 2018). Hence, there is an increasing focus to research and developing host-directed therapies given there is a lower drug resistance potential (Lou et al., 2014). We hypothesized that by combining various influenza interactome studies, there might be novel insights into viral–host interactors and processes that could be targeted for antiviral therapy. In this study, we identified novel host interactors of influenza via literature and database search. We further analyzed the data set by bioinformatics. This resulted in the identification of core cellular processes and druggable targets that could be further studied. This provides an overall landscape of conserved host processes targeted by various influenza strains for future drug development and better understanding the influenza life cycle.

MATERIALS AND METHODS

Data Collection

In order to identify host interactions that are ubiquitous across the various influenza A strains, data was extracted from the Reactome database (Fabregat et al., 2018; Jassal et al., 2020) and Host Pathogen Interaction Database (HPIDB; Ammari et al., 2016) while a PubMed and Scopus search of primary literature that performed interaction studies. Papers were chosen if they had found specific interactions between a host protein and influenza proteins. Host interactions with viral complexes and novel accessory viral proteins, such as PA-X and PB1-F2, were excluded. This is because not all influenza A strains express these accessory proteins. A total of 15 papers were retrieved, and their methodology, as well as virus strain(s) studied, are listed Supplementary Table S1. Reactome is a free, online, curated, open-source pathway database contains the influenza infection pathway (REACT_6167.3), specifically NS1 Mediated Effects on Host Pathways (Homo sapiens). This pathway was last reviewed on 1 May 2007, thus does not contain any information from the studies utilized in this project. Despite its age, it was still included as a point of reference for subsequent analysis. (Fabregat et al., 2018; Jassal et al., 2020).

HPIDB was chosen as it contains a comprehensive set of host–virus interactions (Ammari et al., 2016). This includes experimentally derived HPI, predicted HPI via network analysis, and molecular interactions from other databases which include VirHostNet1 and UniProtKB (Consortium, 2012).2 Currently, in its third version, it has 69,787 unique protein interactions between 66 host and 668 pathogen species. In this project, all ten characterized influenza protein interactions (HA, NA, PA, PB1, PB2, NP, M1, M2, NS1, and NS2) from various influenza A strains with human proteins were extracted from the various studies and databases, compiled, and matched to reveal which are key interactors of influenza. The compiled data can be found in Supplementary Table S1.

Data Set Analysis

Using the filtered gene data set, we performed the following analysis. Enrichr (Chen et al., 2013; Kuleshov et al., 2016) was used for gene set enrichment analysis. Kyoto Encyclopedia of Genes and Genomes (KEGG)3 pathway analysis was conducted

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1http://virhostnet.prabi.fr
2https://www.uniprot.org/
3http://www.genome.jp/kegg/pathway.html
RESULTS AND DISCUSSION

Meta-Analysis of Influenza–Host Interactions

In order to better understand the landscape of influenza–host interactions, we performed an analysis of influenza host interactors. We compiled virus–host protein–protein interaction data from HPIDB, Reactome, and published interactome studies. The published studies and their methodology are described in Supplementary Table S1. This data set covered various strains of influenza A, such as H3N2, H1N1, and H7N9. This data set contained protein interactions from different experimental methods—Affinity purification-mass spectrometry (AP-MS), Yeast 2 hybrid (Y2H), RNA immunoprecipitation, and bioinformatics prediction of interactions. Given that single interactome studies may result in false positives, a host interactor was considered to be true if it appeared in at least 3 different studies and databases. Altogether, our review uncovered 1,717 host interactions among the ten viral proteins. Both KEGG and Enrichr functional analysis were performed for the interactors of each viral protein. Details of the specific host–viral interactions can be found from Supplementary Table S2.

HA Interactors Are Mainly Involved in Protein Processing

HA is a trimer of identical subunits, each containing two polypeptides that result from proteolytic cleavage of a singular precursor (Skehel and Wiley, 2000). This cleavage is essential to activate membrane fusion potential and hence infectivity (Garten and Klenk, 1999; Steinhauer, 1999). A newly synthesized 70kDa HA is cleaved into HA1 and HA2, which are linked by disulfide bonds. HA1 contains the sialic acid binding site. After binding, the virus is internalized into endosomes. Endosomal acidification triggers a marked and irreversible change in HA, which results in the dissociation of HA1 from the endosomal membrane and HA1 moving away from HA2. There is a loop-to-helix transition in HA2 which enables the fusion peptide at the N-terminus of HA2 to attach to the endosomal membrane. This promotes the fusion of the viral and endosomal membranes and this results in the vRNP release into the cytoplasm (Das et al., 2010).

HA binding to sialic acid receptor determines the species-specific infectivity of the influenza virus (Rogers and Paulson, 1983). Avian and equine viruses prefer α-2,3-galactose-linked sialic acid, human viruses prefer α-2,6-linked sialic acid and swine viruses appear to bind to both linkages of sialic acid (Rogers and D’Souza, 1989; Gambaryan et al., 1997; Ito et al., 1998).

Based on our meta-analysis, 36 common host interactors were found across the various studies. The top KEGG pathway identified for HA interactors was the proteasomal pathway (i.e., PSMD6 and PSMD7), protein processing in endoplasmic reticulum (i.e., RPN1, CALR, and PDIA6), and adherens junction (i.e., ACTN1 and ACTN4; Figure 2A; Supplementary Figure S1A). The functional analysis of the interactors revealed that they were mainly involved in the immune pathway (i.e., PSMD6, PSMD7, ACTN4, ARF1, and ANXA2), protein processing (i.e., PSMD6, PSMD7, CALR), and post-translational modification (i.e., PSMD6, PSMD7, CALU, and PDIA6; Figure 2B; Supplementary Figure S1B). Given that HA is being transcribed and translated in the infected cell during the viral life cycle, this would point to the importance of the protein processing being key in influenza replication. Any drug targeting this process would affect the formation of new virions. This is supported by a previous study which showed HA being synthesized by ER-bound biosynthetic machinery and interacting with ER chaperone proteins calnexin and calreticulin (Hebert et al., 1997). Any drug targeting this process would affect the formation of new virions. Moreover, HA requires glycosylation for binding to sialic receptors (de Vries et al., 2010), while palmitoylation of HA is essential for the virus to form infectious virions (Chen et al., 2005). Therefore, this presents a key druggable target for a new therapy to target or prevent influenza infection. DAS181 has already been developed as a sialidase fusion protein.
to prevent the binding of haemagglutinin to sialic acid receptors. It has reached late-stage clinical trials (Koszalka et al., 2017).

An interesting observation was that immune-related processes were highly enriched from the gene set analysis. A previous study had reported that HA subunit 1 drove the IFN receptor chain IFNAR1 degradation, thus suppressing IFN-triggered JAK/STAT. The reduced JAK/STAT activation would lead to lower type I interferon production, resulting in decreased immune response and thereby increasing viral replication (Xia et al., 2016). This would confirm that HA is involved in regulating the host immune response as part of the influenza life cycle.

### NA Interactors Are Involved in Vesicle Transport

NA is a mushroom-shaped protein and is found as a tetramer of identical subunits, with the mushroom head suspended from the viral membrane on a thin, long stalk. Each subunit that forms the mushroom head is made up of a six-bladed propeller-like structure (Varghese et al., 1983).

During viral replication, NA removes sialic acid from cellular glycoproteins and glycolipids, as well as from both viral glycoproteins. This prevents newly assembled viruses from rebinding to the infected cell surface and with self-aggregate through HA-sialic acid interactions. New virions are then released from the cell to infect new cells and further the infection spread (Gamblin and Skehel, 2010). It is also thought that NA aids viral infectivity by breaking down the mucus in the respiratory tract secretions to allow the penetration of the virus to the respiratory epithelium and may play a role in viral entry into respiratory epithelial cells (Matrosovich et al., 2004).

A total of 36 NA interactors were found across the various studies. These proteins are involved in fatty acid metabolism (i.e., TECR and HACD3); focal adhesion tight and adherens junction (i.e., ACTN1 and ACTN4), and cell cycle (i.e., MCM7 and PRKDC) via KEGG analysis (Supplementary Figure S2A). Functional analysis of NA interactors found that the most highly enriched processes were intra-Golgi vesicle transport and vesicle transport (i.e., COPB2, COPA, and COPG1; Figure 2B). Given that acetylation of α-tubulin occurs as part of the viral release (Husain and Harrod, 2011), this would suggest that NA is involved in this mechanism.

### NP, PB1, PB2, PA Interactors Are Involved in Splicosome Activity

NP is a structural protein with no enzymatic activity but is the most abundant viral protein in infected cells (Hu et al., 2017). It is an important part of the vRNP complex and its functions include RNA packing (Eisfeld et al., 2015), nuclear trafficking (Amorim et al., 2013; Chutiwitoonchai and Aida, 2016), and vRNA transcription and replication (Eisfeld et al., 2015). A NP monomer has a molecular weight of 56 kDa that is able to bind to 24 bases of RNA (Hu et al., 2017). It is crescent-shaped with head, body, RNA binding, and tail domains (Cianci et al., 2013). The residues in the basic loop (residues 73–91) were found to be required for RNA binding (Ng et al., 2008). NP oligomerization occurs via a flexible tail-loop (residues 402–428) that can insert into the body domain of a neighboring NP monomer (Ye et al., 2006). This tail insertion is facilitated by R419 and E339 which forms a critical salt bridge for stabilization (Cianci et al., 2013). NP also directly interacts with PB1 and PB2 subunits of the viral polymerase (Biswas et al., 1998; Fodor, 2013; Eisfeld et al., 2015; Davis et al., 2017). The C-terminus of NP (aa 340 to 498) contains a PB2 binding site and a sequence that regulates the NP-PB2 interaction.
In addition to its role in the vRNP, NP has been found to induce apoptosis in host cells (Tripathi et al., 2013; Nailwal et al., 2015) and inhibit PKR activation via Hsp40 (Sharma et al., 2011).

The vRNP polymerase complex is a heterotrimer formed together by PB1 with PB2 and PA in the viral polymerase (Eisfeld et al., 2015). PB1 itself has the polymerase activity and is enclosed by the PA linker on one side (Ma et al., 2017) and the N-terminal domain of PB2 at the other side (Stewart and Naessens, 2016). PA contains the endonuclease domain while PB2 has the cap-binding domain (te Velthuis and Fodor, 2016). The LLFL motif in PB1 N-terminus (residue 7–10) interacts with the PA C-terminus hydrophobic core (F411, M595, L666, W706, F710, V636, and L640; Massari et al., 2016). Based on a crystal structure, the C-terminus of PB1 (residues 678–757) was found to complex with the N-terminus of PB2 (residues 1–37; Sugiyama et al., 2009).

Among the three subunits of the vRNP polymerase complex, PB1 had the least interactome studies and the least number of interactors. The most identified interactor for PB1 was Pp65R3. Among all the studies, PB had 316 interactors while NP had 51 interactors and PB2 had 45 interactors. The interactors of NP, PB2, and PA are mostly involved in spliceosome based on the KEGG pathway (Figure 2A; Supplementary Figures S3A–C). The common process for all the interactors of NP, PB2, and PA is the transportation of proteins into the nucleus (i.e., NCBP1, SRSF1, PHAX, U2AF1, SRRM1, BAG3, UBR5, and IPO5; Figure 2B; Supplementary Figures S3D–G). This is expected as the vRNP is required to enter the nucleus for viral replication. In addition, spliceosome pathway is a common process for the interactors of all the vRNP components (i.e., SF3B4, DDX5, SF3B2, SF3B3, SF3B6, SRSF1, U2AF1, CHERP, TRA2B, DHX15, SRSF3, SRSF6, SRSF7, SF3B1, RBMX, DDX5, FXR2, NCBP1, PCBP1, and SNRPA). These interactors are also involved in general RNA processing (Supplementary Figures S3D–G). This may explain the spliceosome being identified with these host interactors, since the spliceosome is part of the RNA processing pathway (Licatalosi and Darnell, 2010; Wilkinson et al., 2020). This is a unique observation as NS1 has traditionally been the viral protein associated with spliceosome inhibition due to its binding to CPSF4 (Twu et al., 2006; Ramos et al., 2013). It was previously reported that the vRNP complex is required to stabilize the NS1-CPSF30 complex, specifically NP and PA (Kuo and Krug, 2009). However, the role of the viral polymerase complex alone in spliceosome regulation has yet to be studied.

The proteasome pathway was a specific pathway identified for PA interactors (i.e., PSMD6, PSMD7, PSMD4, PSMD2, PSMD3, and PSMD1). This would correlate to other studies which has found that treatment with proteasome inhibitors resulted in an antiviral state in cells (Dudek et al., 2010; Haasbach et al., 2011). It was also reported that treatment with the clinical approved proteasomal inhibitor PS-341 resulted in degradation of 1kB and the activation of NF-κB and JNK/AP-1 pathway (Dudek et al., 2010). Hence, this suggests that the proteasomal pathway may be present a novel method of targeting influenza.

An interesting finding was that Annexin A2 (ANXA2) was identified as an interactor of PA across 3 papers (Bradel-Tretheway et al., 2011; Watanabe et al., 2014; Heaton et al., 2016) and HPIDB. Previously, it was reported that ANXA2 binds to highly pathogenic H5N1 influenza NS1 to enhance viral replication. Moreover, ANXA2 is incorporated into IAV particles to enhance viral replication, via the conversion of plasminogen to plasmin (LeBouder et al., 2008).

### M2 Interactors Are Involved in Fatty Acid Metabolism

The M gene encodes for both M1 and M2 proteins (Lamb et al., 1981). M1 protein consists of 252 amino acids, with 2 domains (N-terminal domain from amino acid 1 to 164 and the C-terminal domain from amino acid 165 to 252) linked by a protease-sensitive loop (Ito et al., 1991). It forms the matrix layer by oligomerizing directly below the lipid envelope and binds the viral ribonucleoproteins. It has the important function of stabilizing the whole envelope structure of a fully formed virion (Harris et al., 2001; Calder et al., 2010; Schaap et al., 2012; Adachi et al., 2017). M1 contacts with both viral RNA and NP, promoting the vRNP complex formation and cause the RNP dissociation from the nuclear matrix (Wakefield and Brownlee, 1989; Elster et al., 1994, 1997; Nasser et al., 1996; Chaimayo et al., 2017). M1 plays an important role in assembly by recruiting viral components to the assembly and an essential role in budding, such as viral particle formation (Gómez-Puertas et al., 2000; Latham and Galarza, 2001). M1 had the least interactome studies and only three common interactors were found: EZRI, HSP70, and STA1. Based on these three interactors, the interactors were found to be positive regulators of virus replication (Figure 2; Supplementary Figures S4A,B).

The M2 protein comprises of 97 amino acids with three domains: extracellular (24 amino acids), transmembrane domain (19 amino acids), and cytoplasmic domain (54 amino acids). It is a membrane protein which is inserted into the viral envelope and projects from the surface of the virus as tetramers (Lamb et al., 1985; Holsinger and Alams, 1991). The M2 protein is a proton channel and is required in the acidification of the viral particle upon endocytosis (Lamb et al., 1985) and prior to membrane fusion to enable the release of vRNPs into the cytosol (Helenius, 1992). It is also required to prevent the Golgi lumen pH from becoming too acidic so that the nascent HA do not undergo premature conformational arrangement while being transported to the plasma membrane (Sugrue and Hay, 1991).

Eighty-nine host proteins were found to interact with M2 across the various interactome studies. The most common interactors were 4F2, AFG32, ECHB, SPTC1, and TMX3. KEGG analysis revealed that these proteins were mainly involved in fatty acid metabolism (i.e., TECR and HACD3) and DNA replication (i.e., RFC3 and MCM7; Figure 2A; Supplementary Figure S4C). Functional enrichment analysis showed that these proteins were involved in vesicle transport (i.e., COPB2, COPA, ZW10, GBF1, and COPG1; Figure 2B;
Supplementary Figure S4D). Given that ubiquitination of M2 is required for viral packaging and release (Su et al., 2018), this would confirm the importance of the vesicle pathway for influenza via M2.

An interesting observation was the involvement of M2 interactors in fatty acid metabolism. Fatty acid oxidation was found to be reduced in influenza-infected mice (Ohno et al., 2020), while supplying palmitic acid increased influenza replication (Limsuwan et al., 2020). Influenza replication could be reduced by a fatty acid import inhibitor (Limsuwan et al., 2020). Hence, this would suggest that M2 may be involved in the fatty acid metabolism dysregulation caused by influenza infection. In addition, a recent study reported that M2 clustering which enables membrane scission is mediated by cholesterol (Elkens et al., 2017). Given that cholesterol is involved in viral membrane fusion, viral genome release, and viral budding, this may explain the efficacy of cholesterol-lowering drugs, gemfibrozil, and lovastatin in reducing the stability and infectivity of progeny virus (Bajimaya et al., 2017). It was also demonstrated that overexpression of Annexin A6 as well as the addition of U18666A, a hydrophobic polyamine, was able to reduce cholesterol levels in the plasma membrane and inhibit viral replication (Musiol et al., 2013).

NS1 Interacting Partners Are Involved in Spliceosome and Autophagy

NS1 is not part of the virion structural component, but it is expressed at high levels in infected cells (Hale et al., 2008). It is composed of 231–237 amino acids, depending on the strain, and has a molecular mass of around 26kDa (Hale et al., 2008). It has two distinct functional domains: an N-terminal RNA binding domain (amino acids 1–73) and a C-terminal effector domain (amino acids 86–231/237), which mediates binding with host cell proteins (Wang et al., 2002; Kochs et al., 2007; Hale et al., 2008; Das et al., 2010). NS1 was reported to have multiple functions that contribute to viral replication and virulence (Kochs et al., 2007; Hale et al., 2008; Fournier et al., 2014). These include: (i) temporarily regulating viral RNA synthesis (Hale et al., 2008; Ayllon and García-Sastre, 2015); (ii) viral mRNA splicing control (Hale et al., 2008, Ayllon and García-Sastre, 2015); (iii) enhancing viral mRNA translation via PKR inhibition (Li et al., 2006); (iv) regulating the creation of the virus particle structure (Hale et al., 2008; Pereira et al., 2017); (v) suppressing the host immune or apoptotic responses (Ehrhardt et al., 2007; Kochs et al., 2007; Iwai et al., 2010; Jia et al., 2010; Mata et al., 2011; Gao et al., 2012; Anastasina et al., 2016); (vi) activating phosphoinositide 3-kinase (Ehrhardt et al., 2007; Gaur et al., 2011; Ayllon and García-Sastre, 2015); and (vii) involvement in strain-dependent pathogenesis (Hale et al., 2008). NS1 exists as a homodimer. The RNA binding domain binds to RNA and the binding is dependent on R38 and other charged residues, such as R35 and K41 (Lalime and Pekosz, 2014; Ayllon and García-Sastre, 2015). In addition, the effector domain of NS1 has been found to bind to CPSF30, which results in reduced IFN-β mRNA production. The key amino acid residue for the CPSF30 interaction is W187 (Engel, 2013). Moreover, multiple mutations in NS1 have been found to increase virulence (Ozawa et al., 2011; DeDiego et al., 2016) and viral pathogenicity (Ehrhardt et al., 2007; Engel, 2013; Nogales et al., 2017).

NS1 had the most interactome studies among all the viral proteins. A total of 252 interactors were found among the interactome studies. The most common interactor found was STAU1 found in nine studies, followed by PRKRA. Based on the KEGG pathway, these interactors are involved in spliceosome (i.e., SF3B2, SNW1, FUS, NCBP2, PCBP1, TRA2B, TRA2A, DHX15, SRSF3, SRSF6, SRSF7, and RBMX) and autophagy (i.e., IRS1, BAD, IRS4, RAF1, and TANK; Figure 2A; Supplementary Figure S5A). Enrichr analysis also revealed that these interacting partners are involved in mRNA splicing (i.e., DDX17, PRDX6, QKI, FXR1, PTBP1, FXR2, SNW1, SON, TRA2B, TRA2A, SRSF3, SRSF6, SRSF7, and RBMX) and RNA processing (i.e., CPSF4, SF3B2, CHTOP, RBM14, CPSF1, FUS, NCBP2, CPSF2, PTBP1, SNW1, SON, PCBP1, TRA2B, TRA2A, DHX15, SRSF3, RALY, SRSF6, SRSF7, and RBMX; Figure 2B; Supplementary Figure S5B). NS1 is a well-known interactor of the spliceosome pathway, given its interactions with CPSF4 and NS1-BP (Wolf et al., 1998; Thompson et al., 2018; Zhang et al., 2018). It has been previously reported that NS1 interacts with hnRNP-F to modulate host mRNA processing (Lee et al., 2010). In addition, NS1 is required for unspliced M1 nuclear export (Pereira et al., 2017). NS1 is also a well-known inducer of autophagy (Zhirnov and Klenk, 2013; Zhang et al., 2019). It was previously reported that NS1 induced autophagy via its interaction with JNK (Zhang et al., 2019).

NS2 Interactors Are Mainly Involved in Focal Adhesion and ECM–Receptor Interaction

NEP or non-structural protein 2 (NS2) is a structural protein and is associated with M1 (Yasuda et al., 1993). NEP mediates vRNP nuclear export into the cytoplasm via an export signal (O’Neill et al., 1998) through XPO1 interaction (Neumann et al., 2000). NEP has also been found to interact with nucleoporins and is suggested to act as an adaptor between vRNPs and the nuclear pore complex (O’Neill et al., 1998). It has been proposed that NEP is involved in the transcription and replication of the influenza virus (Robb et al., 2009).

Forty host proteins were found to be NS2 interactors across the different studies. These proteins are involved in focal adhesion and ECM–receptor interaction (i.e., LAMC3, ZYX, and LAMB1), and ubiquitin-mediated proteolysis (i.e., PIAS3 and SKP1) via KEGG analysis (Figure 2A; Supplementary Figure S6A). Functional enrichment analysis showed that these proteins are involved in microtubule reorganization (i.e., DCTN2, CENPH, and ZWINT) and ER-Golgi transport (i.e., COG8 and COG6; Figure 2; Supplementary Figure S6B).

Influenza Interacting Partners Are Involved in the Spliceosome, Focal Adhesions, and Protein Processing in the ER

A global analysis of the protein interactors revealed that most of these interactors are involved in the spliceosome, followed
by focal adhesion and protein processing in the ER via KEGG analysis. Given the large proportion of NS1 interactome studies, this may have resulted in spliceosome being the top process. However, it was interesting to note that focal adhesion and protein processing in the ER was one of the top processes revealed for influenza interactors.

A previous study revealed that actin reorganization is required for influenza assembly and budding via cofilin-1 phosphorylation (Liu et al., 2014). Focal adhesion kinase (FAK) is a non-receptor tyrosine kinase (non-PTK) and part of focal adhesions that tether actin cytoskeleton to the extracellular matrix. It was previously shown that FAK is involved in phosphatidylinositol-3-kinase (PI3K) activation and reorganization of cytoskeleton for endosomal trafficking during IAV entry (Elbahesh et al., 2014). In addition, FAK was found to positively regulate IAV replication and polymerase activity of different IAV strains (Elbahesh et al., 2016). FAK-dependent regulation of innate immune responses was observed during severe IAV infection in mice (Bergmann and Elbahesh, 2019). Given that HA, NA, and NS2 have interactors involved in focal adhesions or ECM, this may be a potential target for novel influenza therapy.

In addition, a novel finding was the involvement of these interactors in protein processing in the ER. Figure 3 describes the interaction of the influenza proteins and host proteins involved in ER protein processing. It was previously reported that influenza modulates vesicle trafficking via disruption of the Golgi complex (Yadav et al., 2016). In addition, brefeldin A, an ER to Golgi transport inhibitor, was found to affect the intracellular distribution of HA (Russ et al., 1991; Ciampor et al., 1997) and induce apoptosis in influenza-infected cells (Saito et al., 1996). Monensin, a Golgi complex disruptor, was found to reduce viral budding and HA localization to the cell membrane (Edwardson, 1984).

Given the role of influenza proteins, NS1, NP, PA, and PB1 in spliceosome activity, targeting this pathway may be a potential target for influenza. Cdc2-like kinase 1 (CLK1) is a kinase that regulates alternative splicing of pre-mRNA (Bullock et al., 2009). CLK1 inhibition by TG003 or CLK1 knockdown was shown to decrease M2 mRNA generation and downstream M2 protein expression, thus reducing IAV propagation (Karlas et al., 2010).

A total of 190 host proteins had more than 1 viral protein interaction. 4F2 was the most promiscuous host protein with 6 viral protein interactions. ADT3, ANXA2, PSD11, PSD12, PSD13, and TCPE had 5 viral protein interactions each. Given that these proteins have interactions with multiple viral proteins, this may indicate that these proteins are key for multiple aspects of viral replication and life cycle and, hence, should be further studied for novel host-directed therapy.

**CONCLUSIONS AND FUTURE WORK**

Identifying novel host interactors in influenza is key in understanding the viral life cycle as well as for the development of novel therapies against the virus. Our review of influenza interactors has comprehensively compiled influenza interactome studies together with host–virus interaction databases. While other studies have done interactome studies on influenza proteins, no study has done a comprehensive review on influenza interactors. Using interactome studies to derive our data set also enables a more direct virus–host interaction compared to RNAi studies and would include essential genes which may lead to cell death in RNAi studies, thus reducing false negatives (Watanabe et al., 2010). While Watanabe et al. (2010) and Tripathi et al. (2015) both did meta-analysis of RNAi studies, no other study has done a meta-analysis of interactome studies for influenza. Our study has revealed novel influenza interactors which can be potentially targeted for novel therapies against influenza. It also filters out possible false positives that may be derived from a single interactome study. Given that we used a benchmark of at least three separate studies to filter out true positive, this would reflect the sensitivity of our study to detect conserved host interactors of influenza. Moreover, given that our study included various strains of influenza A, this would increase the likelihood that these pathways are globally used by all influenza A strains for their life cycle. Hence, these pathways can be further studied as potential universal therapy against influenza.

Based on our bioinformatics analysis of influenza proteins, we observed that protein transport to the ER is one of the top biological processes exploited by influenza (Figure 2). This supports the previous study by Heaton et al. (2016), where they identified Sec61 knockdown was found to reduce influenza replication. This suggests that the protein transport to the ER is one of the key processes that influenza exploits for its life
cycle. In addition, ER transport is a key process in the innate immunity. A member of the COPII complex, Sec13, was previously identified in a CRISPR knockout screen to reduce influenza replication (Li et al., 2020). This would support the important role of ER processing for influenza. Hence, more studies into how it can be targeted for influenza treatment should be undertaken. Several pro-inflammatory cytokines, such as IL6 and IFN-β, are secreted (Murray and Stow, 2014) via the ER-Golgi pathway. Influenza’s control over this pathway would enable it to replicate without detection from immune cells and this would reflect the importance of the protein transport system. Moreover, influenza’s ability to overstimulate the immune response via cytokine storm has been shown to be correlated to virus virulence (Kido, 2015; Li et al., 2018; Short et al., 2018). This correlates with other RNAi studies performed in influenza which reflect the importance of the ER to Golgi transport pathway in viral replication (Tripathi et al., 2015). Given the importance of post-translational modification of influenza proteins in viral replication and host response, as discussed by Hu et al. (2020), this would be a potential target for influenza treatment, especially in the context of severe influenza. This finding can be extrapolated to other viruses as discussed in the review (Ravindran et al., 2016). Both enveloped and non-enveloped viruses were described to hijack the ER for replication. HIV utilizes the ER to synthesize its envelope glycoprotein (Checkley et al., 2011). Antiviral therapeutics that impair ER-resident glycan trimming enzymes α-glucosidases I and II have been shown inhibit viral infection by DNA and RNA viruses (Chang et al., 2013). Moreover, an inhibitor against HSP70, a cytosolic chaperone that controls ER-associated degradation, has been shown to inhibit flavivirus infection (Taguwa et al., 2015).

Future work would be to validate the targets identified in this review via in vitro and in vivo models. However, this review has reflected the key processes that can be potentially targeted for host-directed therapy against influenza. Given the key role these processes play in influenza as well as normal host cell maintenance, it would be important to find key differences between normal cellular and viral infection. This would enable specific targeting of influenza-driven pathways without killing the host. Another aspect that would need to be studied is how these processes contribute to severe influenza, which is still currently unknown. Therefore, a more detailed analysis of IAV–host interactions would provide clues for therapeutic targeting and molecular mechanisms of viral replication.

**AUTHOR CONTRIBUTIONS**

SC, JC, DE, and LL designed the experiments. SC performed the experiments. SC, JC, and LL analyzed the data. SC wrote the manuscript. DE and LL did the final editing of the manuscript. All authors contributed to the article and approved the submitted version.

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DE holds a Wolfson family chair in Biochemistry.

**SUPPLEMENTARY MATERIAL**

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2022.869406/full#supplementary-material

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