In silico investigation of the viroporin E as a vaccine target against SARS-CoV-2

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Abstract

Viroporins, integral viral membrane ion channel proteins, interact with host-cell proteins deregulating physiological processes and activating inflammasomes. Severity of COVID-19 might be associated with hyperinflammation, thus we aimed at the complete immunoinformatic analysis of the SARS-CoV-2 viroporin E, P0DTC4. We also identified the human proteins interacting with P0DTC4 and the enriched molecular functions of the corresponding genes. The complete sequence of P0DTC4 in FASTA format was processed in 10 databases relative to secondary and tertiary protein structure analyses and prediction of optimal vaccine epitopes. Three more databases were accessed for the retrieval and the molecular functional characterization of the P0DTC4 human interactors. The immunoinformatics analysis resulted in the identification of 4 discontinuous B-cell epitopes along with 1 linear B-cell epitope and 11 T-cell epitopes which were found to be antigenic, immunogenic, nonallergen, nontoxin, and unable to induce autoimmunity thus fulfilling prerequisites for vaccine design. The functional enrichment analysis showed that the predicted host interactors of P0DTC4 target the cellular acetylation network. Two of the identified host-cell proteins – BRD2 and BRD4 – have been shown to be promising targets for antiviral therapy. Thus, our findings have implications for COVID-19 therapy and indicate that viroporin E could serve as a promising vaccine target against SARS-CoV-2. Validation experiments are required to complement these in silico results.

1 INTRODUCTION

The severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2) emerged in China at the end of December 2019. Globally, as of 19 January, 2021, there have been 94,124,612 confirmed cases of the coronavirus disease 2019 (COVID-19) including 929,994 deaths reported to WHO (https://covid19.who.int/). The clinical presentation of COVID-19 is diverse, ranging from asymptomatic infection to mild upper respiratory tract illness to severe interstitial pneumonia with respiratory failure and death (1). A systematic review and meta-analysis of 45 studies with a total number of 4,203 patients reported that the pooled rates of intensive care unit (ICU) admission, mortality, and acute respiratory distress syndrome (ARDS) were 10.9%, 4.3%, and 18.4%, respectively (2).

The COVID-19 disease not only affects the lungs but also has cardiovascular, endothelial, and inflammatory sequelae (3). Hyperinflammation and coagulopathy have been identified as the main contributors to disease extensiveness and death in infected patients (4). Severe cases of COVID-19 have been associated with increased serum levels of several inflammatory cytokines and chemokines, leukocytosis, and lymphocytopenia [reviewed in Merad and Martin (4) and Yap et al. (5)]. The activation of the inflammasome pathway and its contribution in the pathobiology of COVID-19 disease are currently being investigated with possible impacts on therapeutic targeting (5).

Viral ion channels or viroporins are integral viral membrane proteins (6) that have been linked to inflammasomes activation (7). The severe acute respiratory syndrome coronavirus (SARS-CoV) encodes three viroporins, the proteins 3a, E, and 8a of which the first two have been shown to be dispensable for maximal SARS-CoV replication (8). The ion channel activity of the E protein has also been identified as a virulence factor in mouse models (8, 9). The transport of calcium cations through the E protein ion channel has also been identified as a virulence factor in mouse models (8, 9). The transport of calcium cations through the E protein ion channel has also been identified as a virulence factor in mouse models (8, 9).
the E protein – keeping the PDZ-binding motif (PBM) intact – could provide the basis for the development of live-attenuated vaccine and inactivated vaccine (12). Computational approaches for vaccine and drug design – as the one mentioned above – have guided the development of novel therapeutic strategies during the last decade. In silico methodology has widely been used for the identification of drugs and vaccine candidate epitopes against several bacteria and viruses including SARS-CoV-2 (12–19). Most of these studies have focused on the spike proteins and viral RNA-polymerases as potential vaccine and drug targets (12), whereas very few studies involve viroporin E as a vaccine or drug target candidate (12, 14).

Therefore, in this study, we aimed at the complete immunoinformatic analysis of the SARS-CoV-2 E viroporin, the envelope small membrane protein P0DTC4. In addition, we identified the human proteins interacting with P0DTC4 as well as the significantly enriched molecular activities of the corresponding genes highlighting the role of host proteins interacting with SARS-CoV-2 viroporin E in the immunopathophysiology of COVID-19.

**MATERIALS AND METHODS**

**Immunoinformatic Analysis of the Envelope Small Membrane Protein P0DTC4**

The SARS-CoV-2 E protein sequence in FASTA format was obtained from the Universal Protein Resource (Uniprot) database (https://www.uniprot.org/) (entry P0DTC4) (20). The whole protein antigenicity was determined using the VaxiJen v. 2.0 server (http://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html), which enables the classification of antigens solely based on the physicochemical properties of proteins regardless of the sequence length and the need for alignment (21). This initial examination identified P0DTC4 as a probable antigen with a prediction probability equal to
0.5262 (the predefined threshold for the virus model was 0.4). Subsequently, the secondary and tertiary structure of the protein was modeled by the PSIPRED 4.0 (http://bioinf.cs.ucl.ac.uk/psipred/) (22) and PHYRE2 (http://www.sbg.bio.ic.ac.uk/~phyre2/html) (23) servers, respectively. B-cell linear and discontinuous epitopes were predicted by the ElliPro tool (http://tools.iedb.org/ellipro/) of the Immune Epitope Database Analysis Resource (IEDB) (24). The Vaxitop module of the Vaxign system (http://www.violinet.org/vaxign/vaxitop/) was used for the prediction of epitope binding to MHC class I and class II alleles (25). Vaxitop is a vaccine epitope prediction and analysis system based on the principle of reverse vaccinology. The MHC I epitopes were further investigated for the ability to enhance immunogenicity with the class I immunogenicity tool of IEDB (http://tools.iedb.org/immunogenicity/) (26). The antigenicity of the predicted B- and T-cell epitopes was tested via VaxiJen (21) and peptides that were identified as high antigenic were subsequently examined for allergenicity, toxicity, and ability to induce autoimmunity by the AllerTOP v. 2.0 (https://www.ddg-pharmfac.net/AllerTOP/) (27), ToxinPred (http://crdd.osdd.net/raghava/toxinpred/) (28), and Peptide Match (https://research.bioinformatics.udel.edu/peptidematch/index.jsp) (29) servers, respectively. In all of the aforementioned tools, the default parameters were used and where appropriate, Homo sapiens was selected as the host species. Finally, MHC class II binders were also screened for the ability to induce IFN-γ using the predict module of the IFNepitope server (http://crdd.osdd.net/raghava/ifnepitope/) and the motif and support vector machine (SVM) hybrid approach (30).

Identification of the Human Proteins Interacting with the Envelope Small Membrane Protein P0DTC4 and Functional Enrichment Analysis of the Corresponding Genes

The Biological General Repository for Interaction Datasets (BioGRID) database (https://thebiogrid.org/) (31) was accessed for the investigation of the human proteins that interact with P0DTC4. The genes encoding the identified proteins were entered as a list in the ToppFun module of the ToppGene database (https://toppgene.cchmc.org/enrichment.jsp) (32) for the detection of the statistically significantly enriched molecular functions. ToppFun performs functional enrichment of input gene list based on transcriptome (gene expression), proteome (protein domains and interactions), regulome (TFBS and miRNA), ontologies (GO, pathway), phenotype (human disease and mouse phenotype), pharmacome (drug-gene associations), and bibliome (literature co-citation). We repeated the analysis with the g:GOSt tool of the g:Profiler server (https://biit.cs.ut.ee/gprofiler/gost) that relies on ENSEMBL as a primary data source and was recently updated with new data (33). The workflow of the methodology followed in the study is displayed in Fig. 1. The applied computational tools were selected on the basis of software documentation, performance, and citation in previous publications (16, 34). All analyses were performed on May 2020.

### Predicted Linear Epitope(s):

| No. | Chain | Start | End | Peptide | Number of residues | Score | 3D structure |
|-----|-------|-------|-----|---------|-------------------|-------|--------------|
| 1   | _     | 8     | 14  | ETGTLV  | 7                 | 0.83  | View         |
| 2   | _     | 59    | 65  | YSRXNL  | 7                 | 0.786 | View         |
| 3   | _     | 51    | 56  | LVKPGF  | 6                 | 0.586 | View         |
| 4   | _     | 32    | 43  | AILTAIRLCAYC | 12 | 0.549 | View         |

### Predicted Discontinuous Epitope(s):

| No. | Residues | Number of residues | Score | 3D structure |
|-----|----------|--------------------|-------|--------------|
| 1   | G10, T11, L12, T13 | 4 | 0.793 | View |
| 2   | S60, R61, K63, N64, L65 | 5 | 0.79 | View |
| 3   | S50, L51, V52, K53, S55, F56, Y59, V62 | 8 | 0.619 | View |
| 4   | A32, I33, T35, A36, L37, R38, L39, C40, A41, Y42, C43 | 11 | 0.577 | View |
Table 1. The MHC I binding epitopes of the SARS-CoV-2 E viroporin as predicted by the Vaxitope server*

| Peptide                | Length | Antigenicity Score | Allergenicity | Toxicity | Peptide Match-Ability to Induce Autoimmunity |
|------------------------|--------|--------------------|---------------|----------|--------------------------------------------|
| VLLFLAFVVF31           | 11     | 0.2976             | 0.4012        | Probable nonallergen | Nontoxin | No |
| LLFLAFVVF31            | 10     | 0.3211             | 0.5111        | Probable nonallergen | Nontoxin | No |
| FLAVFVVF31             | 10     | 0.3210             | 0.6159        | Probable nonallergen | Nontoxin | No |
| FLAVFV31               | 10     | 0.3205             | 0.5651        | Probable nonallergen | Nontoxin | No |
| FLAVFV31               | 9      | 0.3018             | 0.5308        | Probable nonallergen | Nontoxin | No |
| LLFLAVVVF31            | 9      | 0.2918             | 0.4568        | Probable nonallergen | Nontoxin | No |
| LLFLAVVF31             | 9      | 0.2341             | 0.8144        | Probable nonallergen | Nontoxin | No |
| VTLAILTALR31           | 10     | 0.2176             | 0.8404        | Probable nonallergen | Nontoxin | No |
| LAFVVF31               | 9      | 0.2141             | 0.7976        | Probable nonallergen | Nontoxin | No |
| VTLAILTALR31           | 9      | 0.21055            | 0.6140        | Probable nonallergen | Nontoxin | No |
| SEETGTLIV31            | 9      | 0.2095             | 0.3052        | Probable nonallergen | Nontoxin | No |

*Only results with >0.2 antigenicity score are presented. Bold lines correspond to epitopes which fulfill prerequisites for vaccine design. MHC, major histocompatibility complex.

RESULTS

Immunoinformatic Analysis of the PODTC4 Protein

The secondary structure and the three-dimensional (3-D) model of the PODTC4 protein are presented in Figs. 2 and 3, respectively. The tertiary model was built on the SARS-CoV E protein pentameric ion channel template (PDB: c5·29B) with 99.8% confidence. The PDB file of the constructed, final model was entered in the ElliPro tool which identified four linear and four discontinuous B-cell epitopes (Fig. 4). Out of the four predicted linear peptides, only AILTALRLCYC was found to fulfill the requirements for vaccine design as it was identified as probable T antigen (score 0.7860), 2 nonallergen, and 3 nontoxin. In addition, no match for this peptide was identified in the peptide search database.

The Vaxitope method identified 52 and 36 unique MHC I and MHC II alleles, respectively. The findings pertaining to the remaining analyses with respect to the immunogenicity of the MHC I epitopes, the ability of MHC class II binders to induce IFN-γ, and the allergenicity, allergenicity, toxicity, and ability to induce autoimmunity of all predicted T-cell epitopes are shown in Tables 1 and 2. In total, II T-cell epitopes were found to fulfill prerequisites for vaccine design.

Identification and Functional Genomic Analysis of the Predicted Host-Cell Proteins

Six human proteins [AP3B1: adaptor-related protein complex 3, β 1 subunit; SLC44A2: solute carrier family 44 (choline transporter), member 2; ZC3H18: zinc finger CCCH-type containing 18; CWC27: CWC27 spliceosome-associated protein homolog (Saccharomyces cerevisiae); BRD2&4: bromodomain containing 2&4] were found to form the host interaction network with PODTC4 (Fig. 5). Lysine-acetylated histone binding and acetylation-dependent protein binding were the most significantly enriched molecular activities of the corresponding genes both in the gProfiler and ToppFun tools (Fig. 6 and Table 3).

DISCUSSION

In this study, we aimed at the investigation of the SARS-CoV-2 E viroporin as a potential vaccine target. In the past few years, viral ion channels are in the focus of antiviral research development (6, 35). It has been shown that compounds which block the ion channel activity are effective antiviral drugs both in vitro and in vivo (36). Most recently, the SARS-CoV-2 3a protein was proposed as a feasible therapeutic target against the COVID-19 disease (37). Both the 3a and E proteins of SARS-CoV have been reported to induce the activation of the NLRP3 inflammasome via their viroporin activity, by disturbing the ionic concentration within the cells which results in the generation of reactive oxygen species by the damaged mitochondria, but also independently of it, by stimulating NF-κB signaling which in turn promotes chemokine production [reviewed in Yap et al. (5)].

Table 2. The MHC II binding epitopes of the SARS-CoV-2 E viroporin as predicted by the Vaxitope server*

| Peptide                | Antigenicity Score | Allergenicity | Toxicity | IFN-γ Induction | Peptide Match-Ability to Induce Autoimmunity |
|------------------------|--------------------|---------------|----------|----------------|--------------------------------------------|
| CINIVNSVL31            | 1.4201             | Probable allergen | Nontoxin | Negative | No |
| YCCNIVNSVS31           | 1.3929             | Probable nonallergen | Toxin | Negative | No |
| CCNIVNSVL31            | 1.3710             | Probable nonallergen | Toxin | Positive | No |
| RLCACYCN31             | 1.1243             | Probable nonallergen | Toxin | Positive | No |
| VSYRVRKNL31            | 1.0946             | Probable nonallergen | Toxin | Negative | No |
| KPSFYYSY31R            | 0.9740             | Probable allergen | Nontoxin | Negative | No |
| SVFWKNN31S             | 0.9490             | Probable nonallergen | Nontoxin | Negative | No |
| VFLVLT31A             | 0.9374             | Probable nonallergen | Nontoxin | Negative | No |
| LAILTALR31             | 0.8872             | Probable nonallergen | Nontoxin | Negative | No |
| SFYVYSSYRK31           | 0.8251             | Probable nonallergen | Nontoxin | Negative | No |
| LLFLAFVF31             | 0.8144             | Probable nonallergen | Nontoxin | Positive | No |

*Only results with >0.8 antigenicity score are presented. Bold line corresponds to the epitope which was also predicted to have the potency to induce IFN-γ. MHC, major histocompatibility complex.
The primary amino acid sequence alignment of the 3a proteins from SARS-CoV and SARS-CoV-2 detected a sequence identity equal to 72.4% (11). We focused on the viroporin E protein and analyzed both its secondary and tertiary structure. The high degree of identity (94.7%) between the sequences of the E proteins of SARS-CoV and SARS-CoV-2 (11) were reflected in our results regarding the 3-D structure of the two viroporins as confirmed by the predicted 3-D model of P0DTC4. This finding strengthened the hypothesis that the two proteins have a conserved function, as proteins with high sequence identity and high structural similarity are likely to possess functional similarity and evolutionary relationships (38). Subsequently, we tested the whole P0DTC4 antigenicity. The SARS-CoV-2 E viroporin was identified as a probable antigen. The succeeding immunoinformatics analysis resulted in the prediction of four discontinuous B-cell epitopes along with 1 linear B-cell epitope and 11 T-cell epitopes which were found to fulfill the criteria of safety and effectiveness for vaccine design. It should be noted that at present, T-cell epitope prediction is considered more developed and reliable than that of B-cell prediction mainly due to the fact that the majority of B-cell epitopes are conformational, and thus they cannot be isolated from the protein structure (39).

Our findings indicate that the selected B- and T-cell linear peptides are antigenic, immunogenic, nonallergen, non-toxin, and impotent in inducing autoimmunity. The significance of these results lies in the fact that they provide vaccine candidates which could be tested immediately on experimental animal models, thus minimizing the cost and time for vaccine epitope research and development against the COVID-19 disease.

Deciphering the mechanism by which viral-host protein-protein interactions induce the molecular pathogenesis of infection is key to identifying novel drug candidates for clinical trials (40). It has been reported that the observed differences of SARS-CoV-2 and SARS-CoV in terms of their pathogenicity and epidemiology are likely attributed to the complex interactions between various viral and host factors (17). Hence, in our study, we also aimed at the identification of the human proteins interacting with P0DTC4. This was important as it has been suggested that each viroporin can interact specifically with other viral or cellular proteins (41).

Table 3. The enriched molecular functions of the predicted host-cell proteins interacting with P0DTC4∗

| ID           | Term name                                              | P Value     | FDR B&H | FDR B&Y | Bonferroni | Genes from Input | Genes in Annotation |
|--------------|--------------------------------------------------------|-------------|--------|--------|------------|------------------|--------------------|
| GO:0070577   | Lysine-acetylated histone binding                      | 1.382E-5    | 2.902E-4| 1.256E-3| 5.804E-4   | 2                | 19                 |
| GO:0140033   | Acetylation-dependent protein binding                  | 1.382E-5    | 2.902E-4| 1.256E-3| 5.804E-4   | 2                | 149                |
| GO:0034211   | GTP-dependent protein kinase activity                  | 6.235E-4    | 7.855E-3| 3.398E-2| 2.619E-2   | 1                | 2                  |
| GO:004030    | Modification-dependent protein binding                 | 8.751E-4    | 7.855E-3| 3.398E-2| 3.675E-2   | 2                | 149                |
| GO:0106140   | P-TEFb complex binding                                 | 9.351E-4    | 7.855E-3| 3.398E-2| 3.927E-2   | 1                | 3                  |

∗B&H, Benjamini and Hochberg; B&Y, Benjamini and Yekutieli; FDR, false discovery rate.
Protein acetylation (PA) is one of the commonest posttranslational modifications (46) and has a key role in transcription regulation.

Increasing evidence has supported the significant contribution of PA in airway inflammation through the epigenetic control of inflammatory genes expression [reviewed in Adcock et al. (47) and Ito et al. (48)]. It has been reported that signaling via the NF-κB/RelA transcription factor/BRD4 axis in distal tracheobronchiolar epithelial cells mediates acute inflammation in response to viral patterns (49). Several viruses have been shown to exploit the host’s PA network to ensure their replication (50–52) and maintain or exit latency (46, 53, 54).

Due to the fact that the bromodomain-containing proteins act as epigenetic modulators controlling both cellular functions and the viral life cycle (55), their targeting (especially the targeting of BRD4) via small molecule inhibitors has emerged as a potent therapy for viral infectious diseases (49, 55–58). Our prediction that BRD2 and BRD4 are functional interactors with the PDZD4 protein was recently verified (59), reinforcing the hypothesis that the SARS-CoV-2 infectivity might also affect cellular acetylation. Considering the antiviral activity of BRD4 inhibitors as well as their ability to reduce neutrophilic airway inflammation (49), we propose the experimental validation of their clinical efficacy against the COVID-19 disease.

In conclusion, in this study we have identified vaccine candidates for SARS-CoV-2 as well as host factors that can be inhibited by highly specific compounds. Our findings have implications for COVID-19 therapy prospects and also indicate that the SARS-CoV-2 viroporin E could serve as a promising vaccine target for this devastating disease. A limitation of this study is that it relies on computational methodology, and therefore experimental data are required to validate the in silico estimates. Still, data mining and immunoinformatics have greatly changed the field of vaccinology providing a focused, evidence-directed experimental investigation of limited epitope candidates (39, 60).

### DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

### AUTHOR CONTRIBUTIONS

S.G.Z. conceived and designed research; E.R. performed experiments; E.R. analyzed data; E.R. interpreted results of experiments; E.R. drafted manuscript; K.I.G. and S.G.Z. edited and revised manuscript; E.R. prepared experiments; E.R. analyzed data; E.R. interpreted results of experimental validation of their clinical efficacy for COVID-19 disease. A limitation of this study is that it relies on computational methodology, and therefore experimental data are required to validate the in silico estimates. Still, data mining and immunoinformatics have greatly changed the field of vaccinology providing a focused, evidence-directed experimental investigation of limited epitope candidates (39, 60).

### REFERENCES

1. Li Y, Shi J, Xia J, Duan J, Chen L, Yu X, Lan W, Ma Q, Wu X, Yuan Y, Gong L, Yang X, Gao H, Wu C. Asymptomatic and symptomatic patients with non-severe coronavirus disease (COVID-19) have similar clinical features and virological courses: a retrospective single center study. Front Microbiol 11: 1570, 2020. doi:10.3389/fmicb.2020.01570.
2. Zhang JJJ, Lee KS, Ang LW, Leo YS, Young BE. Risk factors of severe disease and efficacy of treatment in patients infected with COVID-19: a systematic review, meta-analysis and meta-regression analysis. Clin Infect Dis 71: 2199–2206, 2020. doi:10.1093/cid/ciao576.
3. Lingappan K, Karmouty-Quintana H, Davies J, Akkanti B, Harting MT. Understanding the age divide in COVID-19: why are children overwhelmingly spared? Am J Physiol Cell Mol Physiol 319: L39–L44, 2020. doi:10.1152/ajpcell.00183.2020.
4. Merad M, Martin JC. Pathological inflammation in patients with COVID-19: a key role for monocytes and macrophages. Nat Rev Immunol 20: 355–362, 2020. doi:10.1038/s41577-020-0331-4.
5. Yap JKY, Moriyama M, Iwasaki A. Inflammamomes and pyroptosis as therapeutic targets for COVID-19. J Immunol 205: 307–312, 2020. doi:10.4049/jimmunol.2000515.
6. Fischer WB, Hsu HJ. Viral channel forming proteins – modeling the target. Biochim Biophys Acta 1808: 561–571, 2011. doi:10.1016/j.bbamem.2010.05.014.
7. Farag NS, Breitinger U, Breitinger HG, El Azizi MA. Viroporins and inflammasomes: a key to understand virus-induced inflammation. Int J Biochem Cell Biol 122: 105738, 2020. doi:10.1016/j.biocel.2020.105738.
8. Castano-Rodriguez C, Honrubia JM, Gutierrez-Alvarez J, DeDiego ML, Nieto-Torres JL, Jimenez-Guaderno JM, Regla-Nava JA, Fernandez-Delgado R, Verdia-Baguena C, Queralt-Martin M, Cohen G, Perlman A, Aguilella VM, Solà I, Enjuanes L. Role of severe acute respiratory syndrome coronavirus viroporin E, 3a, and 8a in replication and pathogenesis. MBio 9: e02325–e02417, 2018. doi:10.1128/mBio.02325-17.
9. Nieto-Torres JL, DeDiego ML, Verdia-Baguena C, Jimenez-Guaderno JM, Regla-Nava JA, Fernandez-Delgado R, Castano-Rodriguez C, Alcaraz A, Torres J, Aguilella VM, Enjuanes L. Severe acute respiratory syndrome coronavirus E protein transports calcium ions and activates the NLRP3 inflammasome. Virology 485: 330–339, 2015. doi:10.1016/j.viro.2015.08.010.
10. Yoshimoto FK. The proteins of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2 or n-CoV19), the cause of COVID-19. Protein J 39: 198–216, 2020. doi:10.1007/s10390-020-09901-4.
11. Sarkar M, Saha S. Structural insight into the role of novel SARS-CoV-2 E protein: a potential target for vaccine development and other therapeutic strategies. PLoS One 15: e0237300, 2020. doi:10.1371/journal.pone.0237300.
12. Baral P, Pavadai E, Gerstman BS, Chapagain PP. In-silico identification of the vaccine candidate epitopes against the Lassa virus hemorrhagic fever. Sci Rep 10: 7667, 2020. doi:10.1038/s41598-020-63640-1.
13. Dey D, Borkotoky S, Banerjee M. In silico identification of Tretino in as a SARS-CoV-2 envelope (E) protein ion channel inhibitor. Comput Biol Med 127: 104063, 2020. doi:10.1016/j.compbiomed.2020.104063.
14. Jaiswal S, Kumar M, Mandeep S, Singh Y, Shukla P. Systems biology approaches for therapeutics development against COVID-19. Front Cell Infect Microbiol 10: 560240, 2020. doi:10.3389/fcimb.2020.560240.
15. Jakhar R, Kaushik S, Gakkhar SK. 3CL hydrolyase-based multi epitope peptide vaccine against SARS-CoV-2 using immunoinformatics. J Med Virol 92: 2114–2123, 2020. doi:10.1002/jmv.25993.
16. Min YQ, Mo Q, Wang J, Deng F, Wang H, Ning YJ. SARS-CoV-2 nsp1: bioinformatics, potential structural and functional features, and implications for drug/vaccine designs. Front Microbiol 11: 587317, 2020. doi:10.3389/fmicb.2020.587317.
17. Sunita, Singhvi N, Singh Y, Shukla P. Computational approaches in epitope design using RNA binding proteins as vaccine candidate in Mycobacterium tuberculosis. Infect Genet Evol 83: 104357, 2020. doi:10.1016/j.meegid.2020.104357.
18. Zadeh Hosseingholi E, Zarrini G, Pashazadeh M, Gheibi Hayat SM, Molavi G. In silico identification of probable drug and vaccine candidates against antibiotic-resistant Acinetobacter baumannii. Microb Drug Resist 26: 456–467, 2020. doi:10.1089/mdr.2019.0236.
19. UniProt Consortium. UniProt: a worldwide hub of protein knowledge. Nucleic Acids Res 47: D506–D515, 2019. doi:10.1093/nar/gky1049.
TARGETING SARS-CoV-2 E VIROPORIN

21. Doychtchinoia IA, Flower DR. VaxiLen: a server for prediction of protective antigens, tumour antigens and subunit vaccines. BMC Bioinformatics 8: 4, 2007. doi:10.1186/1471-2105-4-4.

22. Buchan DWA, Jones DT. The Psipred protein analysis workbench: 20 years on. Nucleic Acids Res 47: W402–W407, 2019. doi:10.1093/nar/gkz297.

23. Kelley LA, Mezulis S, Yates CM, Wass MN, Sternberg MJE. The Phyre2 web portal for protein modeling, prediction and analysis. Nat Protoc 10: 845–858, 2015. doi:10.1038/nprot.2015.053.

24. Ponomarenko J, Bui HH, Li W, Fusseeder N, Bourne PE, Sette A, Peters B. Ellipro: a new structure-based tool for the prediction of antibody epitopes. BMC Bioinform 9: 514, 2008. doi:10.1186/1471-2105-9-514.

25. He Y, Xiang Z, Molely HB. Vaxigen: the first web-based vaccine design program for reverse vaccinology and applications for vaccine development. J Biomed Biotechnol 2010: 2975605, 2010. doi:10.1155/2010/2975605.

26. Calis JJA, Maybemo M, Greenbaum JA, Weiskopf D, De Silva AD, Sette A, Keshmiri M, Peters B. Properties of MHC class I presented peptides that enhance immunogenicity. PLoS Comput Biol 9: e1003266, 2013. doi:10.1371/journal.pcbi.1003266.

27. Dimitrov DS, Bangov I, Flower DR. AllerTOP v2—a server for in silico prediction of allergens. J Mol Model 20: 2278, 2014. doi:10.1007/s00894-014-2278-5.

28. Gupta S, Kapoor P, Chaudhary K, Gautam A, Kumar R. Open Source Drug Discovery Consortium, Raghava GP. In silico approach for predicting toxicity of peptides and proteins. PLoS One 8: e73957, 2013. doi:10.1371/journal.pone.0073957.

29. Chen C, Li Z, Huang H, Suzuki BW, Wu CH, UniProt consortium. A fast peptide match service for UniProt knowledgebase. Bioinformatics 29: 2808–2809, 2013. doi:10.1093/bioinformatics/btt484.

30. Dhandha SK, Vir P, Raghava GP. Designing of interferon-gamma inducing MHC class-II binders. Biol Direct 8: 30, 2013. doi:10.1186/1745-6150-8-30.

31. Doughtred P, Stark C, Breiktreutz BJ, Rust J, Boucher L, Chang C, Kolas N, O’Donnell L, Leung G, McAdam R, Zhang D, Solma W, Willems A, Coulombe-Huntington J, Chatr-Aryamonti A, Dolinski K, Tyers M. The BioGRID interaction database: 2019 update. Nucleic Acids Res 47: D529–D541, 2019. doi:10.1093/nig/dpy230.

32. Chen J, Bardes EE, Aronov BJ, Jegga AG. TopGene Suite for gene list enrichment analysis and candidate gene prioritization. Nucleic Acids Res 37: W305–W311, 2009. doi:10.1093/nig/gkp427.

33. Raudvere U, Kolberg L, Kuzmin I, Arab T, Adler P, Peterson H, Viljo J. gProfiler: a web server for functional enrichment analysis and conversions of gene lists (2019 update). Nucleic Acids Res 47: W91–W98, 2019. doi:10.1093/nig/dkj369.

34. Mitra D, Pandey J, Jain A, Swaroop S. In silico design of multi-epitope-based peptide vaccine against SARS-CoV-2 using its spike protein. J Biomol Struct Dyn 6: 1–14, 2021. doi:10.1080/07391102.2020.1829092.

35. Scott C, Griffen S. Viroporins: structure, function and potential as antiviral targets. J Gen Virol 96: 2000–2027, 2015. doi:10.1099/vir0.000201.

36. Hyser JM. Viroporins. In: Electrophysiology of Unconventional Channels and Pores. Switzerland: Springer, 2015. p. 153–181. doi:10.1007/978-3-319-20498-8_7.

37. Tozzi A, D’Amato G. SARS-CoV-2 Viroporin: a behind the scenes therapeutic target. Electronic response to: Mahase E. 2020. Covid-19: Remdesivir is helpful but not a wonder drug, say researchers. BMJ: m7998, 2020.

38. Gan HH, Perlav RA, Roy S, Ko J, Wu M, Huang J, Yan S, Nicoletta A, Vafai J, Sun D, Wang L, Noah JE, Pasquali S, Schlick T. Analysis of protein sequence/structure similarity relationships. Biophys J 83: 2781–2792, 2002. doi:10.1016/S0006-3495(02)75287-9.

39. Sanchez-Trincado JL, Gomez-Perezos M, Reche PA. Fundamentals and methods for T- and B-cell epitope prediction. J Immunol Methods 2017: 2680610, 2017. doi:10.1016/j.jim.2017.05.004.

40. Hufsky F, Lamskiewicz K, Almeida A, Arconchera A, Arighi C, Bateman A, et al. Computational strategies to combat COVID-19: useful tools to accelerate SARS-CoV-2 and coronavirus research. Brief Bioinform 22: 642–663, 2021. doi:10.1093/bib/bbaa232.

41. Nieva JL, Madan V, Carrasco L. Viroporins: structure and biological functions. Nat Rev Microbiol 10: 563–574, 2012. doi:10.1038/nrmi8280.