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editorial

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Could PROTACs Protect Us From COVID-19?

The outbreak of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has resulted in the current coronavirus disease 2019 (COVID-19) pandemic, which has infected over 22 million people worldwide as of mid-August 2020 (www.coronavirus.jhu.edu). Effective treatment is urgently needed and in response multiple traditional and default approaches are being actively pursued by pharmaceutical companies and academic institutions in numerous countries. These efforts include development of small molecules inhibitors, the repurposing of existing drugs, and the development of new vaccines. However, the high mortality of COVID-19 and its uncontrolled spread amongst the population demands more innovative thinking and novel therapeutic approaches to treat the disease. In this article, we analyze specific requirements that define potent PROteolysis TArgeting Chimeras (PROTACs), and discuss their potential therapeutic power to target SARS-CoV-2 for both the treatment and prevention of COVID-19.

PROTACs are a new class of therapeutic small molecules

PROTACs, which have quickly evolved and been recognized as a superior alternative to small molecule inhibition for certain disease targets of interest, are unique bifunctional molecules capable of promoting protein degradation. To achieve this function, PROTACs must bring together the protein-of-interest (POI) and an E3 ligase into a three-body complex, aka ternary complex, which promotes lysine ubiquitination of the target protein and consequential proteosomal degradation [1].

Numerous studies have addressed how certain PROTAC features, such as warhead affinity, linker length and chemical composition [2], translate to efficacy. However, recent reports suggest that PROTAC function is dependent upon PROTAC-induced protein-protein-interactions (PPIs) within the ternary complex even more so than ligand affinity, and that such molecular interactions correlate directly with PROTAC efficacy and selectivity [3]. These characteristics of the ternary complex can be summarized as a set of structural and energetic requirements that determine the ''productivity'' of a PROTAC molecule, as defined by targeted protein degradation. We have examined this set of ''requirements'' for the ternary complex; and explored the biological consequences of developing a new effective PROTAC molecule to target SARS-CoV-2.

Ternary complex requirements determine PROTAC productivity

The ternary complex forms when the PROTAC engages both intended targets, the POI and E3 ligase. This binding activity brings the two proteins together and engages them with PROTAC-mediated protein-protein-interactions [4]. For the ternary complex to be productive, the protein interface must have the following *structural* requirements or features: 1) minimization of

https://doi.org/10.1016/j.drudis.2020.08.007

PPIs that cause steric hinderance or electrostatic clashes along the newly formed protein interface [5,6]; 2) maximization of PPIs that promote structural complementarity at the new protein interface [5,6]; and 3) recruitment of the adequate E3 ligase in a proper binding orientation that facilitates target POI ubiquitination [7].

Additionally, a set of *energetic* requirements key to the productivity of the ternary complex have also been described as assessed by thermodynamic parameters, and collectively defined as ''cooperativity'' [8]. Under this description, efficient protein degradation is promoted by a ternary complex whose components ''cooperate'' to yield a favorable free energy change (ΔG). To achieve a favorable free energy change and a productive ternary complex via PROTAC design, one can: a) maximize PROTAC-induced PPIs that increase the structural complementarity at the interface and overall binding affinity of the interactions, promoting a favorable ΔH for the ternary complex [5]; and/or one can b) optimize ligand and/or protein flexibility and solvation of the ternary complex in a way that results in a favorable entropic (ΔS) change for the complex formation [6].

And while the formation of the ternary complex is necessary, it appears that the stability of such complex, as defined by binding affinity, is secondary to correct selection and recruitment of the E3 ligase and POI. In fact, in defining the PROTAC efficacy, it has been shown that adequate recruitment of the E3 ligase supersedes the strength of the ternary complex as only certain binding orientations result in productive protein degradation, irrespective of the strength of the interaction [5–7]. These findings argue that the structural and energetic attributes may be more important than the binding affinity of the ternary complex for an effective PROTAC. Therefore, our analysis and description of these requirements

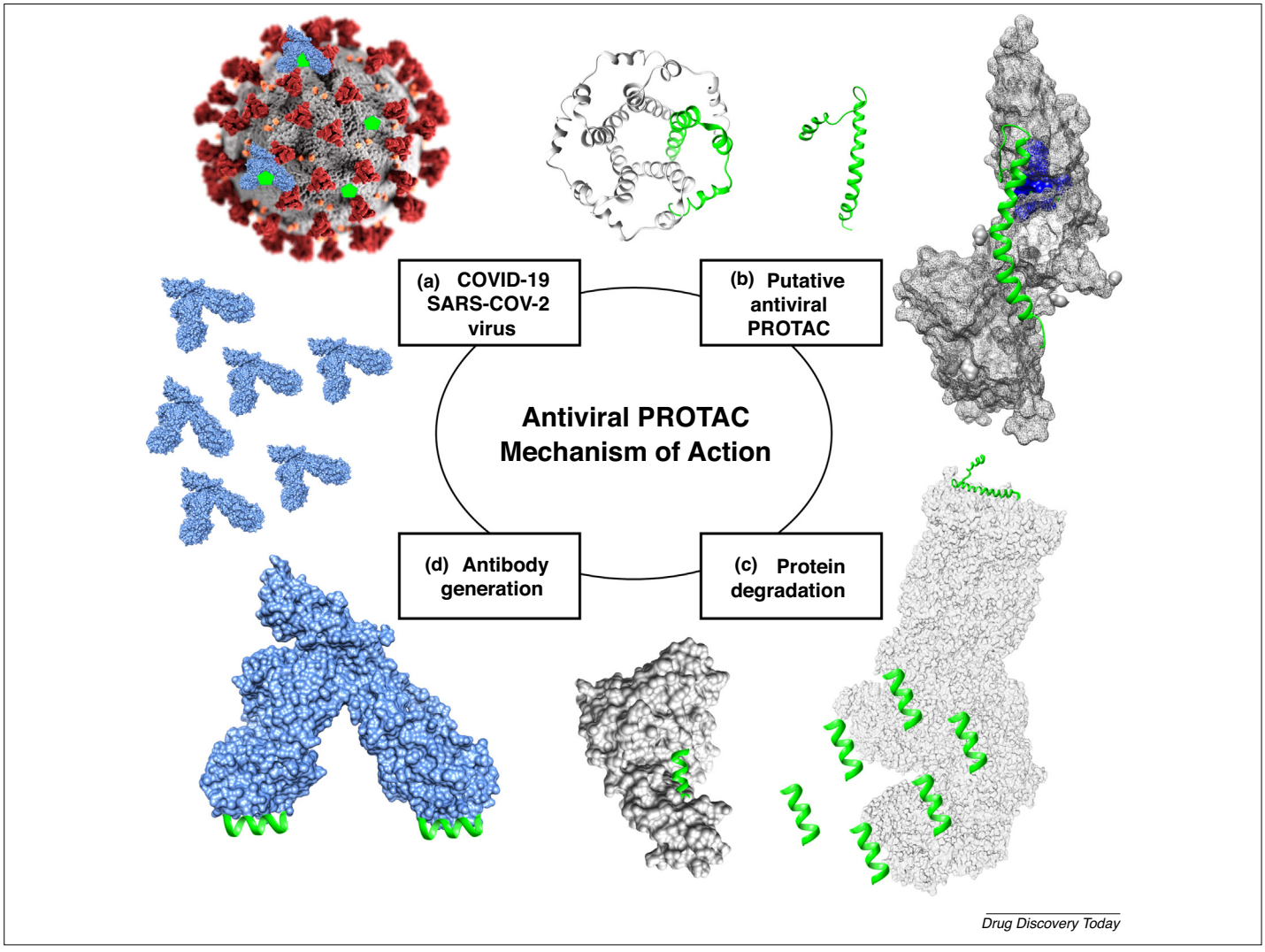

FIGURE 1

Graphical illustration, via molecular structure superimposition, of the mechanism of action of a putative antiviral PROTAC targeting SARS-COV-2 protein E. **(a)** SARS-COV-2 (*illustration adapted from CDC*) highlighing small envelope protein E (green, PDB:5 × 29 pentamer; PDB:2MM4 monomer) as a selected target for PROTAC.

(b) Engagement of a putative PROTAC (royal blue) in a ternary complex with protein E (green) and the targeted E3 ligase (black, PDB:6HAY).

(c) The viral protein E (green) degradation is promoted by the poteosome (gray, PDB:6MSB), and results in the MHC-I presentation of viral epitopes (light gray, PDB:5OQF) to host immune $CD8^+$ T-cells.

(d) Immune response and generation of antiviral antibody (light blue, PDB:4PY8) against SARS-COV-2 protein E in COVID-19.

not only provides a valuable platform for the field, but also postulates a solid starting point to design potent PROTACs for any target of interest.

Development of PROTACs to target SARS-CoV-2

To develop a PROTAC to treat COVID-19 one must select a feasible target protein, such as small envelope protein E within SARS-CoV-2 (Fig. 1**a**). While multiple successful approaches have targeted canonical host proteins in an effort to by-pass rapid mutations of certain viral proteins, targeting viral envelope proteins, such as protein E, has surfaced as a very potent antiviral strategy for coronaviruses such as SARS [9]. Several factors make protein E a unique and feasible target. While the smallest of the three and the one in lower abundance, protein E is the only protein in the viral envelope that is not glycosylated [9]. The lack of glycosylation means that the protein epitopes are not masked by large sugar molecules and readily accessible for small molecule engagement. Functionally, targeting envelope protein E affects virulence, as absence or inactivation of envelope protein can directly affect membrane permeabilizing activity, virion morphology/tropism, viral assembly and virion secretion [9].

An antiviral PROTAC that engages the SARS-CoV-2 protein E in a ternary complex (Fig. 1**b**) and successfully promotes proteasomal degradation (Fig. 1**c**) would abolish all functions of the viral protein and have several unique implications. Indeed, degradation/absence of envelope protein E would result in inhibition of basic viral functions such as viral entry, replication and assembly, which in turn would affect viral multiplication and cell-to-cell infection, essentially diminishing viral load upon infection and potentially preventing severe/chronic viral infection.

Importantly, antiviral PROTACs offer a unique added advantage for the host to develop an antiviral immune response. The innate immune response to viral infection, known as cell immunity, promotes viral clearance and the development of T-cell antibodies, via a mechanism that is directly dependent upon the MHC-I presentation of viral epitopes that are derived from proteasomal degradation of viral proteins [10]. To note is the direct correlation reported between the level of ubiquitination of viral proteins and presentation of viral peptides to MHC-I, where an increase in the levels of ubiquitination of viral proteins, regardless of protein degradation, has been shown to result in an overall increase in the presentation of viral peptides to MHC-I [11]. This suggests that PROTAC-mediated ubiquitination of the viral protein could stimulate the presentation of viral peptides to MHC-I and favor the development of host T-cell therapeutic activity against the viral protein. In fact, this capability has already been reported for PORTACs [4]. These findings mechanistically imply that by leveraging the ubiquitin proteasome system, antiviral PROTACs have the potential of achieving a dual function: 1) degradation of the viral target protein (Fig. 1**c**) and 2) promotion of the generation of antibodies against the viral protein (Fig. 1**d**).

Conclusion remarks

Antiviral PROTACs are orally bioavailable small molecules that can be optimized to have low/null toxicity as well as dual therapeutic function. In principle, oral antiviral PROTACS could be used prophylactically to prevent viral infection in addition to therapeutically to promote protein degradation and host immune response in the case of confirmed disease. Therefore, we propose the use of the design requirements summarized above for the development of new dual-function antiviral PROTACs, as a novel therapeutic strategy that can be deported safely and efficiently to treat and protect the general population against COVID-19.

Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

This work was supported in part by the research funding from the U.S. National Institutes of Health (to M.-M.Z. and W.M.-O.).

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