

EDITORIAL

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Targeting the human TREX complex to prevent herpesvirus replication: what is new?



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Herpesviruses are a leading cause of human viral disease. There are eight distinct herpesviruses known to cause a range of debilitating acute and recurrent diseases in humans [1–3]. Examples include HSV-1, which causes cold sores and genital lesions; hCMV, which is associated with glandular fever and a range of conditions in immunocompromised patients and congenitally infected newborns; and Kaposi's sarcoma-associated herpesvirus (KSHV), which is an oncogenic herpesvirus associated with commonly fatal malignancies in immunocompromised individuals. Currently, drugs in clinical use for the treatment of herpesvirus infections are inhibitors of herpesvirus DNA polymerases. These nucleotide, nucleoside and pyrophosphate analogs are effective against a number of herpesviruses, although drug-resistant strains are emerging in immunocompromised patients [4]. Moreover, poor efficacy has been reported against the oncogenic herpesvirus subfamily [5]. Consequently, there is an urgent need for the continued development of

antiherpesvirus drugs, particularly targeting oncogenic herpesviruses.

Herpesviruses are among the most common viruses found in humans. Once an individual has become infected by a herpesvirus the infection persists, leading to recurrent outbreaks of disease. Herpesviruses have a unique biphasic life cycle, comprising the persistent dormant infection known as the latent state, which can be spontaneously reactivated to initiate the lytic replication cycle. During lytic replication infectious virions are produced, which, in the majority of cases, spread the virus infection, leading to the characteristic disease associated with the herpesvirus. Notably, inhibiting the lytic stages of the herpesvirus life cycle is a key goal for efficacious herpesvirus-targeted therapeutics. Such inhibition, while not eradicating the latent lifelong infection, will treat the pathogenic symptoms associated with herpesvirus infection.

Like all viruses, herpesviruses manipulate and utilize the host cell's machinery to enhance their own replication to produce

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infectious virions, resulting in virus spread and disease. One such example, essential for herpesvirus replication, is the interaction of a conserved family of herpesvirus-encoded RNA-binding proteins with a large cellular multiprotein complex termed TREX [6–10]. Human herpesviruses utilize multiple components of hTREX to stabilize and thus preferentially promote nuclear export of herpesviral mRNAs [11]. Specifically, hTREX serves as a binding platform for the cellular mRNA export factor Nxf1 on herpesvirus mRNAs forming a stable and export competent viral ribonucleoprotein particle.

The recent discovery that a core component of hTREX, the RNA helicase UAP56, remodels the hTREX complex in an ATP-dependent manner [12,13] has given rise to the idea that the interaction of hTREX with herpesvirus-encoded adapter proteins might also be regulated in an ATP-dependent manner. This strategy provided an especially attractive option for inhibition of herpesvirus replication, as targeting of the ATPase function of viral and cellular RNA helicases has already been explored, showing selective pharmacological targeting is possible [14–16]. Notably, the targeting of a cellular RNA helicase would also reduce the risk of viral resistance and would have potential pan-herpesvirus activity.

In a recent publication, the novel approach of targeting hTREX to prevent herpesvirus lytic replication is demonstrated [17]. Using co-immunoprecipitation assays we first identified that ATP-dependent remodeling of hTREX affects the interaction of the KSHV-encoded adapter protein, known as ORF57, independently of the native hTREX complex. Specifically, formation of the complete hTREX required binding of ATP by UAP56. However, this ATP-bound state was insufficient to recruit the KSHV ORF57 protein. Here, a further ATP hydrolysis event was essential to mediate the ORF57–hTREX interaction. We therefore speculated that a small molecule inhibitor disrupting UAP56-mediated ATP hydrolysis would allow an assembly of endogenous hTREX, but prevent KSHV ORF57 recruitment. This, in turn, would prevent herpesvirus viral ribonucleoprotein particle formation and subsequently viral lytic replication. Importantly, as hTREX recruitment is not specific to KSHV but conserved in other human herpesviruses, any inhibitory compound targeting the activity of the cellular UAP56

protein should also inhibit lytic replication in a potentially pan-herpesvirus fashion.

A virtual high-throughput screening approach was therefore used to identify potential inhibitors of UAP56 ATPase activity. Following *in vitro* screening, the prioritized hit compound, CCT018159, was shown to specifically disrupt the interaction of multiple herpesviral adapter proteins, KSHV ORF57, HSV-1 ICP27 and hCMV pUL69, with UAP56, without disrupting endogenous hTREX assembly. Furthermore, the compound inhibited KSHV viral mRNA, but not cellular bulk mRNA nuclear export. Moreover, CCT018159 relieved cells from ORF57-imposed hTREX sequestration, resulting in reduced KSHV DNA replication and infectious virion production. Similar inhibition was also observed for HSV-1 and hCMV lytic replication and infectious virion production.

Our results suggest that a clear therapeutic window, which inhibits herpesvirus replication with limited host cell toxicity, can be achieved. However, CCT018159 is also a known HSP90 inhibitor [18], and although we could effectively inhibit UAP56 activity within HSP90 functional parameters, CCT018159-mediated inhibition of HSP90 at higher concentrations restricted the therapeutic window. This highlights the need for further generation of a more targeted UAP56 inhibitory compound with reduced off-target effects. Here, future medicinal chemistry approaches will endeavor to increase the affinity of CCT018159-related compounds for UAP56, as well as improve its complex pharmacokinetics, which include a relatively high metabolic turnover, and reduce the above-mentioned HSP90-related off-target effects. However, the identification and subsequent characterization of CCT018159 against KSHV, HSV-1 and hCMV has shown that it is possible to target the hTREX complex to inhibit herpesvirus lytic replication. Although we are still several generations from a feasible drug in clinical use, this can be seen as a starting point for lead compound development.

One obvious limitation of this novel targeting approach to treat herpesvirus-related diseases includes, for example, EBV latency-associated lymphomas and carcinomas, and other diseases associated with herpesvirus latent gene expression. Interestingly, however, UAP56 has also been implicated in mRNA processing of other distinct groups of viruses, such as influenza A virus and HBV. While influenza A virus is

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believed to require a virally encoded adapter protein to bridge the interaction between viral mRNA and hTREX [19], HBV employs a special cellular adapter protein [20]. This allows for the intriguing idea that targeting hTREX ATP-dependent remodeling may also be effective at inhibiting not only herpesviruses, but other distinct classes of viruses.

In summary, our study has highlighted a novel approach to target herpesvirus lytic replication and potentially other human pathogens through inhibition of the hTREX complex. While the identified inhibitor is only a hit compound, which now requires a lengthy hit-to-lead development process to produce a compound fit for clinical use, the findings are extremely encouraging, as they demonstrate movement toward a new generation of antiherpesvirus drugs.

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