Structural blueprint for next-generation mpox therapeutics

Zhenzhen Zhou,[1] Weiwei Zou,[1] Xinyong Liu,[1,*] and Dongwei Kang[1,*]

[1]Department of Medicinal Chemistry, Key Laboratory of Chemical Biology (Ministry of Education), State Key Laboratory of Discovery and Utilization of Functional Components in Traditional Chinese, School of Pharmaceutical Sciences, Cheeloo College of Medicine, Shandong University, Jinan 250012, China

*Correspondence: xinyongl@sdu.edu.cn (X.L.); kangdongwei@126.com (D.K.)

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Mpox is caused by the monkeypox virus (MPXV) and is characterized by fever, rash, and lymphadenopathy. The World Health Organization (WHO) has twice declared mpox a Public Health Emergency of International Concern (PHEIC)—first in 2022 and again in 2024—highlighting the urgent need for effective treatments. Although the small-molecular orthopoxvirus inhibitor tecovirimat has been approved for the treatment of Mpox, it has not demonstrated significant efficacy in patients infected with MPXV clade I strains.[1] By contrast, neutralizing antibodies represent a promising strategy, as they can intercept viral entry and cell-to-cell transmission. MPXV produces two virion forms, mature virions (MVs) and extracellular virions (EVs), which are responsible for host-to-host infection and cell-to-cell transmission, respectively.[2] The recent studies by Guardado-Calvo et al., Coelho et al., and Zhang et al. collectively provide the proof of concept for antibody protection targeting the key viral proteins—the fusion complex A16/G9 on MVs and the spread mediator A35 on EVs.[3-5] Their structural biology studies and protective efficacy evaluation *in vivo* confirmed A16/G9 and A35 as therapeutic targets, and offered promising drug candidates for MPXV.

BLOCKING THE ENTRY OF MPXV: STRUCTURAL SECRETS OF THE A16/G9 COMPLEX

Guardado-Calvo and his colleagues focus on the entry-fusion complex (EFC) on the envelope of MPXV MVs, which was composed of 11 highly conserved viral transmembrane proteins. EFC plays a crucial role in the process of virus fusion with the host cell membrane. Unlike other enveloped viruses, the EFC of MPXV can interact with a fusion-suppressive complex,

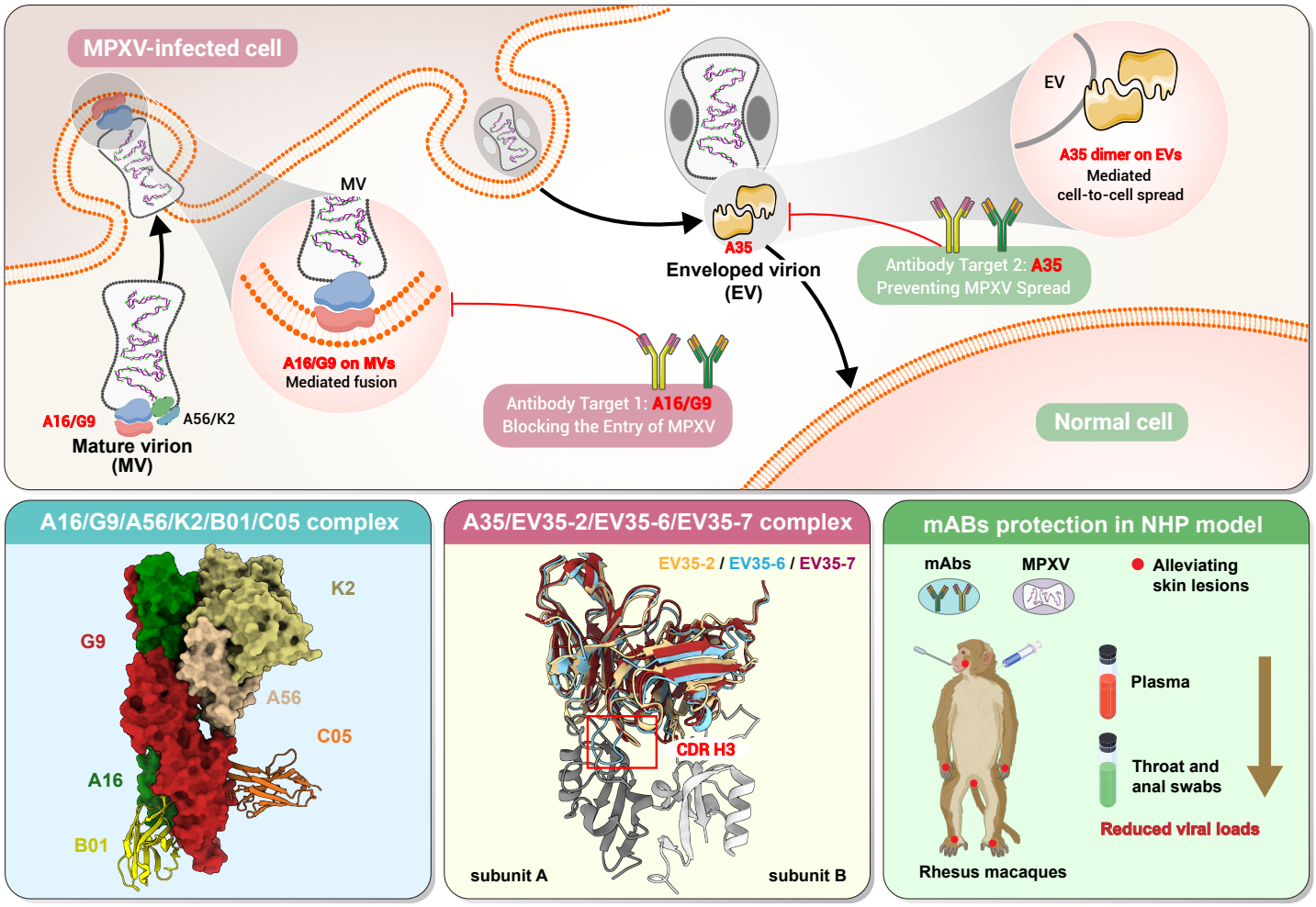

Figure 1. The roles of subcomplex A16/G9 and protein A35 in MPXV infection, and the antibody-related research conducted by Guardado-Calvo, Coelho, Zhang, and their colleagues Structures of A16/G9/A56/K2 complex (PDB: 9HBK), A16/G9/B01/C05 complex (PDB: 9HLS), A35/EV35-2 complex (PDB: 9MSN), A35/EV35-6 complex (PDB: 9MSO), and A35/EV35-7 complex (PDB: 9MSP).

composed of the membrane protein A56 and the serine-protease inhibitor K2, to modulate viral fusion, thereby preventing the formation of superinfection and syncytium (Figure 1). Guardado-Calvo *et al.* provided the first structural basis of the interaction mechanism between A56/K2 and the A16/G9 heterodimer of the EFC (Figure 1). Their work is of great significance as it reveals a precise auto-regulatory mechanism: the A56/K2 binds to the N-

terminal domains (NTD) of A16/G9, inhibiting the fusogenic activity of the EFC at neutral pH, thereby preventing untimely fusion and syncytia formation. Moreover, they found that the NTD of A16 contains a conserved hydrophobic pocket that could adapt the N-myristoyl groups of A16 and G9 to accelerate membrane positioning and improve membrane fusion efficiency, which represents a novel and attractive target for broad-spectrum antiviral design.

They further immunized an alpaca with the recombinant A16/G9 complex, yielding four variable domains of heavy-chain-only antibodies (VHHs), with B01 and C05 demonstrating potent cross-neutralizing activity. Structural biology studies showed that B01 binds to a quaternary epitope at the A16/G9 interface and C05 binds to the C-terminal domain (CTD) of G9. Notably, their structure-based antibody design of the bispecific molecule A56/K2/C05-Fc is a clever strategy, essentially creating a "super-inhibitor" with improved potency by fusing the C05 and the A56/K2 to a human Fc domain. Nevertheless, prophylactic use of A56/K2/C05-Fc failed to protect mice from the VACV infection, despite reducing viral load in lungs. This may suggest that anti-MVs antibodies require a combination with the antibodies targeting EVs to provide full protection.

HALTING VIRAL TRANSMISSION: TARGETING MPXV WITH HUMAN A35-NEUTRALIZING ANTIBODIES

Compared with the single lipoprotein membrane of MVs, EVs have an additional membrane that helps them escape from recognition by the immune system and spread between cells. Coelho and Zhang coincidentally turned their attention to MPXV EVs, and believed that the critical protein A35 on EVs represents a promising target for therapeutic antibodies.

From the memory B cells of a convalescent MPXV patient, Coelho et al. successfully identified three human monoclonal antibodies (mAbs), named EV35-2, EV35-6, and EV35-7. They could bind to MPXV A35 and its vaccinia virus ortholog A33 with picomolar affinity, and effectively prevent the cell-to-cell spread of VACV *in vitro*. Significantly, prophylactic administration of any of the three mAbs provided 100% protection against lethal VACV attack, and EV35-6 could still rescue most mice with an administration after 24 hours of infection. Notably, the post-exposure efficacy of EV35-6 is particularly promising, as it aligns with the real-world clinical need to treat patients after symptom onset.

Mechanism studies showed that the mAbs acted through a dual pathway, Fc-dependent and Fc-independent. The Fc-dependent mAbs could recruit immune cells to inhibit the cell-to-cell spread of MPXVs through antibody-dependent cell-mediated cytotoxicity (ADCC) or complement-dependent cytotoxicity (CDC). Significantly, they found that EV35-6 and EV35-7 could still provide potent neutralization without the Fc-mediated function region, which suggested a potential benefit for immunocompromised individuals. Besides, EV35-2, EV35-6, and EV35-7 use the same "CxGGDCx" motif encoded by the same gene fragment (IGHD2-21) in their functional segment CDRH3 loops, which could insert deeply into the A35 dimer binding pocket (Figure 1). This motif may be the first choice provided by our immune system for MPXV and VACV. Finally, the observed correlation between antibodies targeting this conserved A35 epitope and improved patient outcomes is compelling evidence, as it directly validates this target and antibody approach in humans, bridging the gap between animal models and clinical reality.

A CRITICAL STEP TOWARD CLINICAL TRANSLATION: A35 ANTIBODIES DEMONSTRATE STRONG EFFICACY IN A NONHUMAN PRIMATE MODEL

Although Coelho and her colleagues found the association between anti-A35 antibodies and improved patient outcomes, Zhang et al. offer more straightforward evidence that anti-A35 antibodies could provide protection in humans by demonstrating protective efficacy not only in mouse models but also in a non-human primate (NHP) model. Their findings showed that a single dose of mAb 975 or 981 markedly reduced viral loads and attenuated disease severity in a rhesus macaque model, supporting their potential as practical therapeutic candidates (Figure 1). In addition, the establishment of the NHP model represents a significant contribution to the field, providing a credible and standardized system for evaluating future mpox therapeutics.

The high-resolution cryo-EM structures demonstrated that both mAbs bind to the same binding pocket on the A35 dimer as reported by Coelho. Notably, they found that this binding pocket was conserved in the VACV A33 protein, which could be recognized by the murine-derived mAb A27D7. This structurally conserved characteristic reveals that the pocket could be a promising site for the design of broad-spectrum antibodies against orthopoxviruses.

Guardado-Calvo, Coelho, Zhang, and their colleagues jointly outlined the blueprint for antibody therapy of MPXV infection. Their efforts focus on two key pathways in virus infection and transmission. The first one inhibits viral entry through targeting the A16/G9 complex on the MVs, and the other blocks cell-to-cell spread via targeting the A35 protein on the EVs. Both antibodies targeting A16/G9 and A35 provided strong protection against MPXV infection in mice. Specially, antibodies targeting A35 have been proven effective for the first time in the NHP model. Notably, these antibodies have the potential for further development, offering new hope for the treatment of mpox. In addition, all three studies provided high-resolution structure complexes of antibodies with A16/G9 or A35, which revealed the mechanism of their mAbs. The identification of conserved epitopes in the structural studies, such as the N-terminal region of A16/G9 and the dimeric groove of A35, laid the foundation for developing new broad-spectrum antibodies and vaccines. Overall, their studies provided two promising targets and candidates for the development of mopx antibody drugs, and accelerated their clinical applications.

Despite the positive results in the current studies, two interesting concepts have been inspired and need to be proven. Firstly, referring to the cocktail therapy of antiviral drugs, the combination of MV-targeting and EV-targeting antibodies may provide stronger protection against MPVX. Alternatively, the design of a bispecific antibody encompassing the A16/G9 and A35 binding motifs presents an alternative approach to confer complete protection. Secondly, the structural identification of conserved epitopes on both A16/G9 and A35 provides a basis for designing novel broad-spectrum antibodies or next-generation vaccines against orthopoxviruses.

In summary, the studies by Guardado-Calvo, Coelho, Zhang, and their colleagues have brought the discovery of anti-mpox antibodies into the precise design stage driven by structural biology, accelerating the development process of anti-mpox antibody drugs. These foundational discoveries are rapidly being translated into a formidable shield against current and future poxviral threats.

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AUTHOR CONTRIBUTIONS

All authors contributed to and approved the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.