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Predicting the spatial structure of membrane protein and B-cell epitopes of the MPXV_VEROE6 strain of monkeypox virus

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ABSTRACT

By targeting the membrane (M) proteins of monkeypox virus (MPXV) strain VEROE6, we analyzed its evolutionary hierarchy and predicted its dominant antigenic B-cell epitope to provide a theoretical basis for the development of MPXV epitope vaccines and related monoclonal antibodies. In this study, phylogenetic trees were constructed based on the nucleic acid sequences of MPXV and the amino acid sequences of M proteins. The 3D structure of the MPXV_VEROE6 M proteins was predicted with AlphaFold v2.0 and the dominant antigenic B-cell epitopes were comprehensively predicted by analyzing parameters such as flexible segments, the hydrophilic index, the antigenic index, and the protein surface probability. The results showed that the M protein of MPXV_VEROE6 contained 377 amino acids, and their spatial configuration was relatively regular with a turning and random coil structure. The results of a comprehensive multiparameter analysis indicated that possible B-cell epitopes were located in the 23–28, 57–63, 67–78, 80–93, 98–105, 125–131, 143–149, 201–206, 231–237, 261–270, 291–303, and 346–362 amino acid segments. This study elucidated the structural and evolutionary characteristics of MPXV membrane proteins with the aim of providing theoretical information for the development of epitope vaccines, rapid diagnostic reagents, and monoclonal antibodies for monkeypox virus.

1. Introduction

Monkeypox is caused by the monkeypox virus, which has been reported in several countries, is spreading globally, and is causing great panic in the context of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic [1]. On May 7, 2022, the first case of human monkeypox virus (hMPXV) was discovered in tourists returning from Nigeria, which was confirmed by the UK Health Security Agency [2]. On September 16, 2022, the first case of hMPXV infection was reported in the Mainland of China [3]. The hMPXV was first discovered in 1970 and was mainly prevalent in rural rainforests in Central and West Africa. The MPXV genome consists of linear double-stranded DNA (approximately 197 kb) [4]. In September 2017, Nigeria experienced the largest MPX outbreak

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in West Africa. Most of the confirmed cases of the monkeypox epidemic were in adults aged between 21 and 40 years, with a male-to-female ratio of 2.5:1 [5,6]. According to serological data, MPXV outbreaks have occurred in various mammalian species in the region [7]. MPXV is regularly introduced into human populations, as relatively short DNA strands ($\leq$7) may cause human-to-human transmission [8–11]. As of August 19, 2022, more than 40,000 cases had been reported worldwide [12].

MPXV has the same morphological characteristics as those of other poxviruses, and its morphology shows that the viral particles have a brick-like or oval structure [13] and are surrounded by an outer membrane of geometrically corrugated lipoprotein. Morphologically, the prototypical virus shares the typical rectangular shape and size (200 $\times$ 250 nm) of other known poxviruses [14]. The morphological pathogenesis of MPXV involves entry, fusion, replication, and release of the virus. There are two forms of infection—mature virus (MV) and extracellular virus (EV). This is a multi-step process that involves the attachment of the virus to the host cell, fusion of the virus and host membrane, and entry of the core into the cytoplasm [15]. A lipoprotein envelope on the outer layer of MV encloses the viral core and a side body containing certain proteins. MV is released when cells are lysed and are relatively stable in the external environment. It is mainly used for transmission between animals. EV is released through exocytosis and is formed by a lipid membrane around MVs. It originates from the transport Golgi apparatus or endosomes [16]. Although smallpox vaccines provide substantial cross-protection, there is currently no specific treatment or vaccine for monkeypox [17].

Antigen epitopes are the smallest units that the structurally and functionally necessary for the binding of antigens to the corresponding receptors, and they are also the key molecules for the specific recognition of antigens by immune cells, so the presence of antigenic epitopes can stimulate the body to generate immune responses and express the corresponding antibodies to protect the body. Antigenic epitopes determine the specificity of antigenic molecules, which can specifically activate the immune response and target the stimulation of the immune response; hence, the screening of B-cell epitopes plays an important role in the development of next-generation vaccines [18].

For the preparation of vaccines, small-molecule proteins are easier to rapidly express in large quantities, and epitope vaccines can achieve higher safety than that of attenuated vaccines, so the identification of antigenic protein epitopes is important for the development of peptide vaccines and novel drugs [19]. Epitope vaccines are safer than live attenuated and dead vaccines [20]. Epitope recognition with high-performance immunoassay tools is very useful in various applications in the field of epitope mapping, including in peptide-based vaccine design, the recognition of immune processes, the prediction of epitopes for disease diagnosis, the determination of antibody characteristics in various diseases, and the determination of epitopes for disease diagnosis [21–23]. Therefore, accurately inferring the specific amino acid sequences of B-cell epitopes and expressing the corresponding genes in order to achieve the industrial production of low-toxicity immunogenic peptides can be a new means of preparing diagnostic reagents, epitope vaccines, and monoclonal antibodies.

In this experiment, the nucleotide sequence of the M proteins of the MPXV_VEROE6 strain was analyzed, and the spatial structures of the M proteins' amino acid sequences and the dominant antigenic epitopes of B cells were analyzed and predicted. In combination with various factors, the appropriate dominant antigenic B-cell epitopes were screened. This work lays the groundwork for the development of MPXV_VEROE6 epitope vaccines and rapid diagnostic reagents and for the preparation of monoclonal antibodies in order to provide a reliable approach to the development of new and reliable antiviral drugs for countering the regional and even global spread of monkeypox virus.

2. Methodological details

2.1. Materials

The M protein amino acid sequence (GenBank ID: USE04822.1) of the MPXV_VEROE6 strain (GenBank ID: ON754987.1), which was isolated in Europe's Slovenia, broke out at the same time as the COVID-19 and for which each amino acid region has been delineated, was obtained from the NCBI database (https://www.ncbi.nlm.nih.gov/).

2.2. Whole genome sequence analyses

Full-length sequences of the whole genome from the NCBI database were analyzed in this study (Supplementary Table S1). Multiple sequence alignment (MSA) was conducted using the MEGA5.05 software using the ClustalW Multiple Alignment Algorithm. The similarity matrix of these whole genome sequences was computed using the Maximum Composite Likelihood model. Each sequence was aligned to the reference sequence ON754987.1.

2.3. Evolution analysis of poxvirus M protein gene of different species

The M protein amino acid sequence of different poxviruses in different countries and regions in the NCBI database was analyzed by the neighbor-joining method [24]. in MEGA5.05 software (Supplementary Table S2). The phylogenetic tree of the M protein amino acid sequence of poxvirus was constructed, and the genetic distance between different strains was analyzed by MegAlign software. Each sequence was aligned to the reference sequence USE04822.1 (Virion membrane protein A16).

2.4. Prediction of the three-dimensional (3D) structure of MPXV_VEROE6 strain M protein

The M amino acid sequence of the MPXV_VEROE6 strain was used as the research object. AlphaFold v2.0 software provided by the

parallel supercomputing cloud service was used to predict the 3D structure of the protein. In addition to specifying the location of the necessary public data set, parameter max_template_date was set as "2022-09-01". Set parameter model_preset to "monomer" recommended for single-chain prediction, and parameter is_prokaryote_list to "true" to use the prokaryote algorithm for prediction, and Chimera software was used to simulate its 3D structure.

2.5. Analysis of secondary structure and antigenic epitope of MPXV VEROE6 strain M protein

The Protean module was used to analyze the M of MPXV_VEROE6 strain in DNAStar software. The steps are as follows: First, the secondary structure (Garnier-Robson method and Chou-Fasman method), flexible segment (Karplus-Schulz method), and surface possibility (Emini method) of M were predicted. Then, the hydrophilicity of M protein (Kyte-Doolittle method) was analyzed, and its antigenic index and epitope index was predicted (Jameson-Wolf method and Kolaskar-Tongaonkar method, respectively) (http://tools.immuneepitope.org/tools/bcell/iedb_iMut). Finally, the B cell dominant antigen epitopes of MPXV_VEROE6 strain M protein were comprehensively predicted by referring to the 3D structure of the M protein.

2.6. T lymphocyte epitopes prediction

From the hMPXV M protein, cytotoxic T lymphocyte (CTL) epitope binding predictions were performed using the IEDB analysis resource (TepiTool [25]) using the NetMHCpan method [26]. The IEDB SMM method (http://tools.iedb.org/mhci/) was used to predict the IC50 value of the shortlisted epitopes with their respective HLA supertype alleles. Helper T lymphocyte (HTL) epitopes were predicted from the full-length sequences of the selected viral proteins using the NetMHCIIpan 4.0 server [27].

3. Results

3.1. Analysis of the evolution of MPXV_VEROE6

The nucleotide sequences and amino acid sequences of the M proteins of MPXV strains from different countries were separately analyzed, and a phylogenetic tree was constructed by using the neighbor-joining method (step size: 1000, boot-strap value: >20%). Analysis with the MegAlign software showed that the nucleotide sequence of the MPXV strains isolated from different countries and regions were very different (6–100%) (Fig. 1 A,Supplementary Fig.S1). The results showed that the most primitive populations in terms of transmission were found in Africa and had evolved in different directions, while in Europe, transmission was rapid and consistent [28]. This suggests that MPXV is also spreading globally in the context of the COVID-19 pandemic. The nucleotide sequences of the MPXV strains from different countries and regions were very different, indicating that MPXV evolved in different directions under the pressure of different hosts. There was little difference in the amino acid sequences of the M proteins of the MPXV strains from the different countries and regions (92–100%) (Fig. 1 B), indicating that the evolution of the M protein sequences of the MPXV strains was relatively conservative.

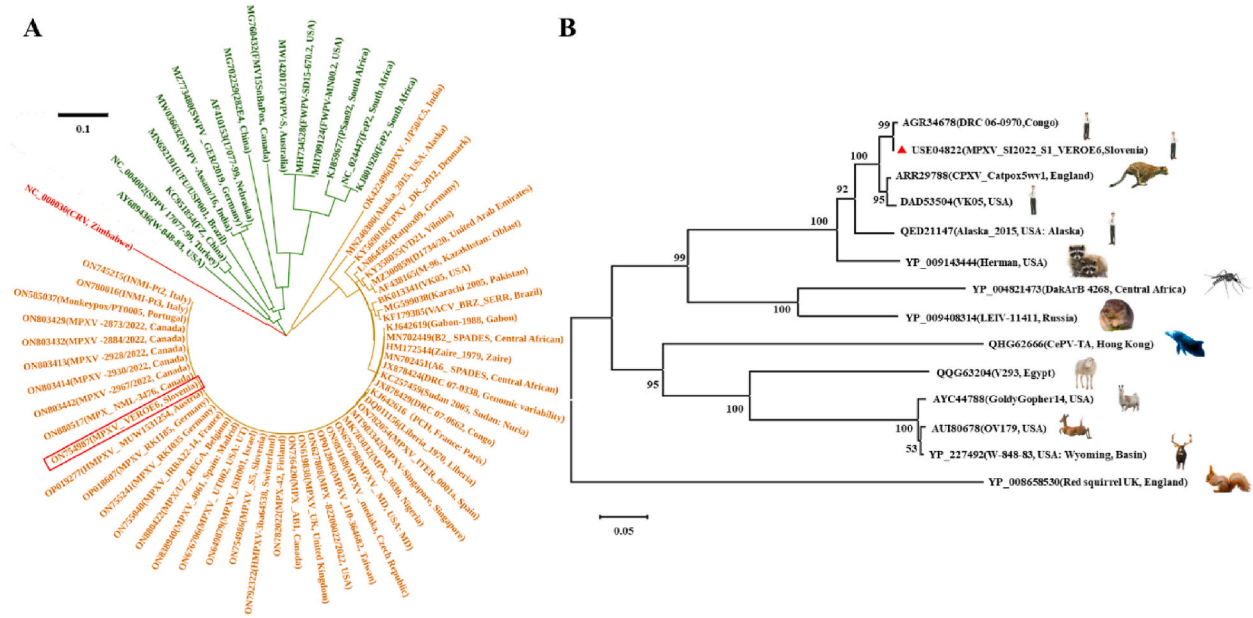

Fig. 1. Phylogenetic tree of the nucleotide sequences and M proteins of MPXV. (A) Phylogenetic tree of the MPXV nucleotide sequence isolated from different countries and regions; (B) phylogenetic tree of the MPXV M protein amino acid sequences isolated from multiple hosts.

3.2. Prediction of the 3D structure of M protein

The 3D structure of the amino acid sequences of the M proteins of the MPXV_VEROE6 strain was simulated with AlphaFold v2.0 and analyzed with the Chimera software. The results showed that the spatial structure of the proteins was irregularly folded; the α-helix was mostly located in the middle region, the N-terminus was mostly β-folded, the β-turn was basically distributed in the middle posterior region, and the α-helix anchors it onto the surface of the viral particle to provide stable structural support (Fig. 2 A, B). The amino acid sequence of the transmembrane topology of the M protein of the MPXV_VEROE6 strain was predicted by using YRC Philius Server (https://www.yeastrc.org/philius/showResults. do), and the results showed that most of its structure was an extramembrane structure (Fig. 2 C).

3.3. Secondary M protein structure of MPXV_VEROE6

Two methods were used to analyzed the secondary structure of M protein, and the final results supported each other. The α-helix predicted with the Garnier-Robson method was mainly distributed in the middle and upper segments of the amino acid sequence. There were α-helixes that spanned the largest region (10 aa) in the 1–10, 34–43, and 206–215 segments. The large number of β-sheets were more evenly distributed; the largest section was 336–360 (25 aa). The distribution of β-turns was also uniform, with the largest β-turns occurring in the 251–259 region (9 aa) in the presence of a single amino acid. The prediction results of the Chou-Fasman method and Garnier-Robson method overlapped in some areas: α-helixes were dispersedly distributed, and there was an α-helix (12 aa) across the largest area in the 186–197 section. β-sheets wers is dispersedly distributed, and the maximum was 23 aa in the 339–361 section. The β-turn areas were evenly distributed (Fig. 3, Supplementary Tables S3 and S4). The flexible regions of the M protein of the MPXV_VEROE6 strain predicted with the Karplus-Schulz method were distributed in 9–14, 20, 21, 31–33, 41, 42, 49–58, 87–92, 94–99, 105–110, 113–116, 124–127, 132–134, 138–142, 148–154, 161–167, 177–184, 192–195, 199–201, 222, 223, 229, 238–243, 250–262, 271–277, 283–291, 305–322, 325–332, 334–341, and 364–374. It can be seen that the M protein had multiple flexible regions and could form multiple dominant epitopes (Fig. 4).

3.4. Hydrophilicity and surface potential of M protein of the MPXV_VEROE6 strain

The hydrophilicity (Kyte-Doolittle method) analysis showed that the M protein's hydrophilic region accounted for a relatively high proportion that covered almost all regions, further proving that the protein was hydrophilic (Fig. 5). Analysis of the surface probability (Emini method) showed that the 8, 10, 19–22, 29–35, 40–51, 53–56, 78, 79, 93, 94, 96, 103–108, 110–118, 131–134, 150–154, 159, 161–167, 179–181, 190–196, 199, 208, 209, 220, 223, 239–242, 250–257, 259, 260, 270–287, 289, 290, 306–309, 317, 319, 335–338, 362–368, and 373–377 areas of the surface were the most likely (Fig. 6). The results of the analysis indicated that the repeated parts predicted with the two methods were the most likely to be located on the M surface and have the characteristics of viral antigenic epitopes.

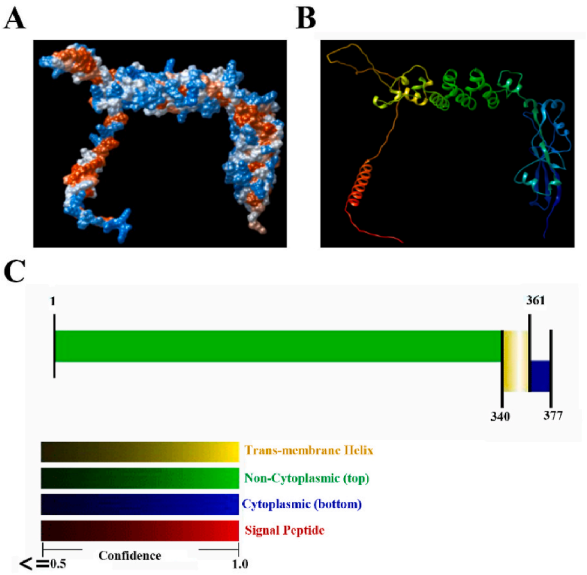

Fig. 2. Virion membrane protein A16. (A) Predicted 3D structure of the M protein amino acid sequence of the MPXV_VEROE6 strain; (B) spatially folded conformation of the M protein amino acid sequence of MPXV_VEROE6 strain; (C) YRC Philius Server predicted the spike (non-cytoplasmic) structure of the M protein of the MPXV_VEROE6 strain.

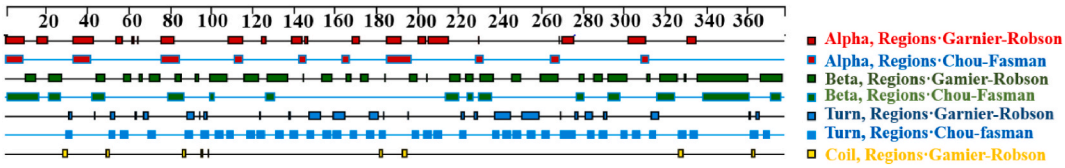

Fig. 3. Prediction of the secondary M protein structure of MPXV_VEROE6 with different methods.

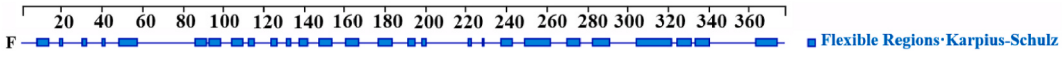

Fig. 4. Prediction of the flexible section of the M protein of MPXV_VEROE6 with the Karplus-Schulz method.

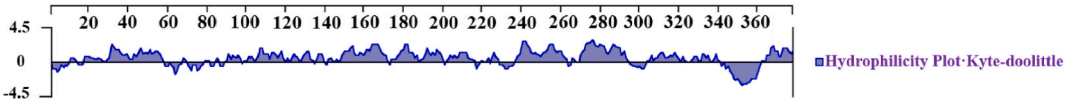

Fig. 5. The Kyte-Doolittle method was used to predict the M protein's hydrophilicity in MPXV_VEROE6.

3.5. Antigenic index of the M protein of the MPXV_VEROE6 strain

The prediction results of the Jameson-Wolf method showed that several regions with higher antigenic indexes were distributed in the MPXV_VEROE6 M protein, including the 10–12, 18–21, 30–35, 40, 41, 50–57, 69, 87–99, 107–111, 113, 116, 117, 125, 126, 132–143, 147–154, 162–169, 176–185, 190–196, 207–209, 220, 222, 223, 237–243, 251–261, 270–279, 284–293, 307–309, 314–320, 326–338, 362–371, and 373–377 regions, of which 251–261 had the highest antigenic index and was the most likely to be a dominant epitope (Fig. 7). The prediction results of the Kolaskar-Tongaonkar method showed that the antigenic index of the M protein of the MPXV_VEROE6 strain fluctuated between 0.883 and 1.245, with an average value of 1.034 (Fig. 8).

3.6. Selection of CTL and helper T lymphocyte cell epitopes

The protein sequences were submitted to the IEDB (NetMHCpan BA 4.1 method) (http://tools.iedb.org/mhci/) to predict CTL ($CD8^+$ T cell) and helper T lymphocyte ($CD4^+$ T cell) epitopes, respectively. $CD8^+$ T cell epitopes were predicted from hMPXV M proteins using the NetCTL 1.2 tool (Supplementary Table S5). We then predicted 74 potential $CD4^+$ T cell epitopes (15-mer) from the antigenic MPXV M protein that could interact with at least three unique HLA-DRB alleles (Supplementary Table S6).

3.7. Comprehensive analysis of the B-cell epitope prediction results for the MPXV_VEROE6 M protein

First, the antigenic epitope parameters such as segment flexibility, hydrophilicity index, antigenic index, and protein surface possibility of the M protein sequence of MPXV_VEROE6 were analyzed. Then, regarding the results for the antigenic epitope index, this study predicted that if a certain segment met the conditions of antigenic index $\geq$1.034 (mean), surface possibility $\geq$1.12 and hydrophilicity $\geq$0, contained at least six amino acid residues, and there was a flexible segment in the internal or adjacent region, this segment could be used as a B-cell epitope of the M protein. In combination with the results of the spatial prediction, a total of 12 dominant epitopes that fulfilled the above conditions were screened (Table 1).

4. Discussion

Recent sporadic outbreaks of novel MPXV in several non-endemic countries have raised major concerns about the distribution of monkeypox in the global population in the context of a global COVID-19 pandemic. The smallpox vaccine is considered the most effective way to control MPXV. Research has shown that the vaccine strain (MVA-BN) and MPXV-2022 have ten different proteins [29, 30], thus suggesting the importance of developing vaccines for against the latest circulating strains. Muhammad Waqas et al. designed a multivalent antigenic epitope vaccine against hMPXV and its variants by fusing information from strains isolated from different

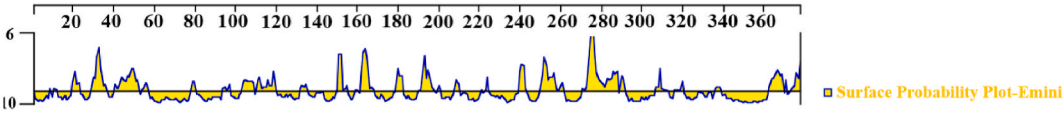

Fig. 6. The Plot-Emini method was used to predict the M protein's surface probability in MPXV_VEROE6.

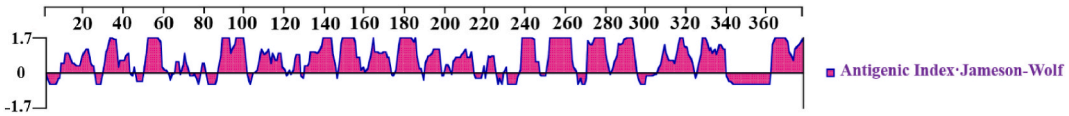

Fig. 7. The Jameson-Wolf method was used to predict M protein's antigenic index in MPXV_VEROE6.

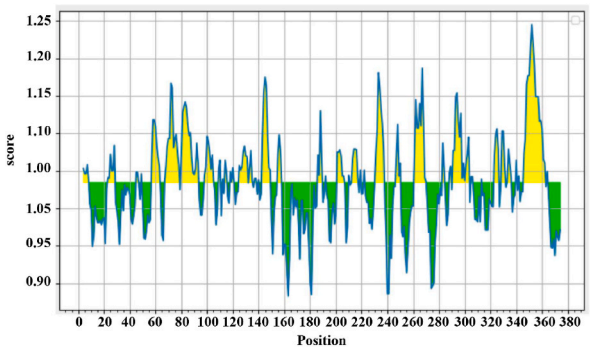

Fig. 8. The Kolaskar-Tongaonkar method was used to predict the M protein's epitope index in MPXV_VEROE6.

Table 1
The amino acid sequence of the M protein B cell epitope in MPXV_VEROE6.

Sequence number	Initial position	Terminal position	Amino acid sequence of B-cell antigenic epitope	Length (aa)
1	23	28	YMVLDF	6
2	57	63	PKFCLID	7
3	67	78	IDHCSSFIVPEF	12
4	80	93	KQYVLIHGEPCSSF	14
5	98	105	GSLIYYQN	8
6	125	131	GQRCHFI	7
7	143	149	VAKCCSK	7
8	201	206	SDICSK	6
9	231	237	ALYVFCN	7
10	261	270	GPRVCWLHEC	10
11	291	303	CKYVGCTINVNSL	13
12	346	362	SITLVVISVIFYFISIY	17

regions using an immunoinformatics approach, targeting extracellular proteins and using the cell membrane as a conjugate [31]. Accurate prediction of the M-antigenic epitopes of the MPXV_VEROE6 strain can provide direction for future work.

When a living organism encounters a pathogenic virus or microbe, the immune system's B cells recognize the pathogen's antigens through membrane-bound immunoglobulin receptors, and the perfect peptide vaccine can induce long-lasting humoral immunity, which is the same natural immune response produced by a pathogenic infection. B cell epitopes stimulate humoral immunity, which can inhibit an infection by producing antibodies against human-exposed antigens [32]. Antibodies must exert their immune effects under the condition that the shape and size of their antigen-binding sites match the antigenic epitope [33]. B-cell epitopes support the design of peptide-based constructs that can be used as vaccine components, as an accompaniment to immunodiagnostics to monitor anti-peptide antibody responses after vaccination, and as a means of producing anti-peptide antibodies. In addition to mediating protective immunity, these constructs can also be used as immunodiagnostic reagents for antigen detection [34]. Two previously approved drugs for smallpox, tecovirimat and brincidofovir, were effective against the monkeypox virus in vitro and in animals [35, 36]. However, given the global emergency, it is necessary to develop specific antibodies that can be used to rapidly detect and prevent MPXV.

This study used a new comprehensive multiparameter prediction method to improve accuracy. The results showed that the mean value of the M protein's antigenic index was 1.034, the regions with high antigenic epitope indexes were evenly distributed, and the maximum value was 1.245. The 23–28, 57–63, 67–78, 80–93, 98–105, 125–131, 143–149, 201–206, 231–237, 261–270, 291–303, and 346–362 amino acid regions covered most of the regions with high antigenic indexes, indicating that these regions may have potential as epitopes.

Hopp-Woods pioneered the hydrophilic parameter prediction method [37,38], which laid the foundation for antigenic epitope prediction. Epitope antigenicity depends on the interaction of multiple factors rather than any single condition due to the polymorphism of antigenic epitopes. B-cell epitopes should be located on the protein's surface and should have a certain degree of flexibility. To bind with antibodies or lymphocyte surface ligands more accurately, the antigen conformation must change to a certain

extent, and it must be mostly flexible. In addition, the biological characteristics of antigenic epitopes are also highly correlated with their secondary and tertiary structures. Studies have shown that the cell-surface-binding protein is one of the major proteins involved in the pathogenesis of MPXV. Then the most promising epitopes can be selected by using a strict procedure, and this can be used for vaccine design [39]. The findings reported in our work will help develop appropriate treatments and facilitate the development of future anti-MPXV vaccines, and this could be an important milestone in producing antiviral vaccines for MPXV.

To solve the problem of antigenic complexity, a method based on multiple prediction of MPXV B-cell antigens was proposed in this study. The current study has some limitations, and it is unclear how well the vaccine obtained from this prediction model protects against MPX infection. In addition, there are various challenges in obtaining vaccine methods through bioinformatics prediction, such as standard benchmarks, limited prediction methods, and the scarcity of datasets for various computational studies [31,40]. Therefore, epitope vaccines generated based on multiple predictions need to be experimentally validated using in vitro and in vivo bioassays to demonstrate their safety and efficacy.

In conclusion, an evolutionary analysis of the nucleotide sequence of the MPXV_VEROE6 strain and the amino acid sequence of the M protein was carried out in this study, and the secondary structure, flexibility, hydrophilicity, surface possibility, antigenic index, and other parameters of the M protein were separately analyzed; then, the tertiary structure prediction results were used to screen the epitopes. Finally, the dominant B-cell epitopes of the M protein were determined through multi-dimensional screening of all of the above parameters. The results of this study provide a reference for further elucidating the structural characteristics of the M protein and further exploring the invasion and pathogenesis of the monkeypox virus to help solve a series of problems caused by this infection. Further, we suggest that future studies use a combination of multiple parameters to predict antigenic epitopes. This would enable rapid screening for antibodies with prophylactic or therapeutic effects to rapidly limiting the widespread spread of infectious viruses and diseases.

Author contribution statement

Zhiyuan Lv; Feng Ji: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Jianzhong Song; Ming Chen: Performed the experiments.

Panpan Li: Analyzed and interpreted the data.

Junmin Chang: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.

Data availability statement

Data will be made available on request.

Declaration of competing 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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e20386.

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