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Comparative Structural and Functional Analysis of Viral Protein E3L and Human PKR dsRBM

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Abstract

Objective: This study undertakes a bioinformatics analysis to compare the structures of the double-stranded RNA-binding motif (dsRBM) of the host protein kinase R (PKR) with that of the viral protein E3L from various poxviruses. The objective is to establish a foundational understanding of PKR's antiviral mechanism and to inform drug design strategies. Methods involved selecting and retrieving E3L and PKR sequences from genome databases, followed by an analysis of their evolutionary relationships through sequence alignment and phylogenetic tree construction. The dsRBM structures of E3L and PKR were examined in both humans and zebrafish to investigate their molecular mechanisms and evolutionary systematics. Results from the sequence alignment of the dsRBMs of PKR and E3L in humans and zebrafish revealed that the aligned sequences were 67 amino acid sequences in length. Notably, the viral E3L and the third dsRBM of zebrafish PKR exhibited high conservation, with numerous conserved residues. It is hypothesized that E3L may be involved in the heterodimerization of the third dsRBM of zebrafish PKR, with the conserved amino acids critical for heterodimerization primarily located at positions 3, 5, 7, 8, 35, 37, 50, 51, 53, 56, 57, 58, 60, and 64. Phylogenetic tree analysis further suggested an evolutionary relationship between PKR and the viral protein E3L. It was also found through molecular docking software and scoring algorithms that its molecular structure has a high degree of similarity. **Conclusion:** The molecular spatial models of the dsRBM of viral protein E3L and human PKR both exhibit an *α*-*β*-*β*-*β*-*α* folding state. Despite the significant differences in their amino acid sequences, the molecular structures of viral protein E3L and PKR are highly similar. Based on this, it can be speculated that the two have a highly similar evolutionary origin.

Keywords

PKR, E3L, Structural Comparison, Functional Analysis, Molecular Structure

1. Introduction

E3L is one of the major interferon (IFN) resistance genes encoded by the vaccinia virus, featuring a highly conserved carboxyl-terminal double-stranded RNA binding domain (dsRBD) and consisting of 190 amino acids [1]. The dsRBD is essential for IFN resistance and virus-related phenotypes across a broad range of hosts [2]. Several studies have shown that the N-terminal of E3L is involved in the indirect regulation of PKR activation, Z-DNA binding, and nuclear localization. Although the N-terminal of E3L is not required for replication and IFN resistance in RK13 and HeLa cells, its deletion in animal models reduces pathogenicity [3] [4].

PKR is one of the most extensively studied components in cellular antiproliferative responses and antiviral defense mechanisms [5]. It is activated upon binding to double-stranded RNA (dsRNA), including viral transcripts. PKR then immediately promotes its own phosphorylation and dimerization in an RNA sequence-independent manner. Activated PKR phosphorylates the translation initiation factor eIF2α as well as the NF-κB inhibitor IκB [6]. Phosphorylation of eIF2αinhibits the guanine nucleotide exchange factor eIF2B, thereby suppressing protein synthesis at the translation initiation stage, a process associated with antiviral defense [7]. The PKR protein is composed of two characteristic domains: the N-terminal dsRNA-binding domain (dsRBD) and the C-terminal kinase activity domain [8]. During viral replication in cells, the dsRNA produced binds to the N-terminal dsRBM of PKR and activates PKR, while the C-terminal kinase domain of PKR binds to the substrate protein eIF-2α, phosphorylating it at serine 51 [9]. According to related studies, the dsRBM of zebrafish PKR can only homodimerize, whereas the viral protein E3L can heterodimerize with PKR's dsRBM. Since PKR has at least two dsRBMs, determining which dsRBM E3L heterodimerizes with can be achieved by sequence alignment in this study, identifying the conserved amino acids that determine heterodimerization. This will help understand the structure of PKR and the viral protein E3L, further analyze the mechanism of PKR inhibition and evolutionary process, and use bioinformatics to compare the dsRBM binding domains of PKR and E3L, analyzing their differences and relationships in evolutionary terms. It helps in the design and development of antiviral drugs and has profound significance for the effective prevention and control of viruses (such as smallpox virus, adenovirus, influenza virus, etc.).

2. Methods

2.1. Literature Retrieval and Sources

Using the Chinese and foreign language databases of Nanchang University Library, keywords "PKR" and viral protein "E3L" were entered to select literature that meets the thematic requirements and obtain the full texts. Databases searched include CNKI, PubMed, Nucleotide Database, China Journal Full-text Database, Amino Acid Database, and Chinese Biomedical Literature Database.

2.2. GenBan Serial Numbers of PKR and E3L

Find the GenBank accession numbers related to PKR and E3L from NCBI, as shown below (Table 1):

Table 1. PKR and E3L gene accession numbers and NCBI search URLs.

Name	GenBan serial number	Website
Danio rerio PKR	NP_001107942.1	https://www.ncbi.nlm.nih.gov/protein/NP_001107942.1
Snow trout PKR	AQM74582.1	https://www.ncbi.nlm.nih.gov/protein/AQM74582.1
Homo sapiens PKR	AAF13156.1	https://www.ncbi.nlm.nih.gov/protein/AAF13156.1
Pig PKR	NP_999484.1	https://www.ncbi.nlm.nih.gov/protein/NP_999484.1
Rattus norvegicus PKR	NP_062208.1	https://www.ncbi.nlm.nih.gov/protein/NP_062208.1
Mus musculus PKR	NP_035293.1	https://www.ncbi.nlm.nih.gov/protein/NP_035293.1
Gallus gallus PKR	NP_989818.1	https://www.ncbi.nlm.nih.gov/protein/NP_989818.1
Anas platyrhynchos PKR	ALF04449.1	https://www.ncbi.nlm.nih.gov/protein/ALF04449.1
Squirrelpox virus E3L	YP_008658450.1	https://www.ncbi.nlm.nih.gov/protein/YP_008658450.1
Variola virus E3L	CAA48985.1	https://www.ncbi.nlm.nih.gov/protein/CAA48985.1
Goatpox virus E3L	P21605.1	https://www.ncbi.nlm.nih.gov/protein/AFX59019.1
Myxoma virus E3L	QDP38495.1	https://www.ncbi.nlm.nih.gov/protein/QDP38495.1
Buffalopox virus E3L	AFI49529.1	https://www.ncbi.nlm.nih.gov/protein/AFI49529.1
Swinepox virus E3L	P32225.1	https://www.ncbi.nlm.nih.gov/protein/418187
Yokapox virus E3L	AEN03626.1	https://www.ncbi.nlm.nih.gov/protein/AEN03626.1
Deerpox virus E3L	ABI99198.1	https://www.ncbi.nlm.nih.gov/protein/ABI99198.1

2.3. Screening the Amino Acid Sequences of PKR and E3L in the NCBI Database

Search for the amino acid sequences of PKR and E3L on NCBI, then perform a BLAST search with the retrieved amino acid sequences on NCBI to screen a series of amino acid sequences. Process the qualified AA sequences using Word software to convert them into the .txt format required by ClustalX2 software, the .aln format required by BioEdit software, and the. fasta format required by MEGA software.

2.4. Sequence Alignment

The method for sequence alignment is as follows: Using the ClustalX2 software to align 16 amino acid sequences that meet the requirements, thereby obtaining the alignment results. The purpose of aligning the 8 PKR and 8 E3L amino acid sequences in this study is to identify certain similarities between the PKR and E3L sequences, then determine the conserved sites among the sequences, and finally analyze the regions where differences exist between the sequences. The specific steps are as follows:

1) Copy the PKR and E3L amino acid sequences that meet the requirements from NCBI into a Word document for editing, removing any spaces, paragraph marks, and numbers.

2) Copy the edited PKR and E3L amino acid sequences into a Notepad TXT file and save it with the format "> Name AA Sequence".

3) Open the ClustalX2 software, click on "Load Sequences" under the file menu, open the TXT file saved in step 2, then click "Do Complete Alignment" under the Alignment menu to perform a full amino acid sequence alignment.

4) Compare each type of PKR and E3L as well as the full gene sequences and nucleotide sites one by one to identify conserved sites, variable sites, and high affinity points.

2.5. Construct a System Phylogenetic Tree

A phylogenetic tree refers to a branched diagram in which biologists and evolutionists place various organisms based on the closeness of their evolutionary relationships, succinctly illustrating the evolutionary process and relationships among species. In a phylogenetic tree, each leaf node represents a species, and if each edge is assigned an appropriate weight, the shortest distance between two leaf nodes can represent the degree of difference between the corresponding two species.

This analysis is based on the amino acid sequences of PKR and E3L to construct a phylogenetic tree. The MEGA software is used to build the phylogenetic tree to study the structural and evolutionary relationship between PKR and the viral protein E3L. The main steps are as follows:

1) Sequence alignment: Save the alignment results from the ClustalX2 software mentioned above as a .aln file.

2) Format conversion: Open the BioEdit software, click "Open File" in the "File" menu to open the .aln sequence alignment file, then click "Delete Sequences" in the "Edit" menu to remove the last line, and finally save it as a fasta file.

3) Constructing a Phylogenetic Tree: Open the MEGA program, click the "File" menu, and select "open a file/session…". In the dialog box, locate the prepared clustal.fa file and open it. A dialog box will appear, select "align" to perform the alignment. The sequence alignment window will open; select all sequences and choose the "Align by ClustalW" option from the Alignment menu to perform multiple sequence alignment using the ClustalW program. A parameter setting dialog box will pop up; use the default parameters and press "OK" to start the

alignment. After the alignment is complete, choose the Data menu, then Export Alignment -> MEGA format to export the multiple sequence alignment result in MEGA format and save it as clustal.meg for later use. Close the sequence alignment window. Return to the MEGA main window, click the “Phylogeny” button, and choose a method for constructing the phylogenetic tree, selecting the NJ algorithm. Open the previously generated clustal.meg, and the “Analysis Preferences” dialog box will appear, where you can set parameters for phylogenetic tree analysis. Usually, modify the “Test of Phylogeny” option to the “Bootstrap method” and then change “No. of Bootstrap Replications” to 1000, meaning the number of resampling repetitions is 1000. All other settings remain as default.

2.6. Molecular Simulation of Spatial Structures

Input the amino acid sequences of PKR and E3L predicted by SMART into the Phyre2 protein 3D structure online system for corresponding 3D molecular spatial simulation. Compare the spatial molecular simulation diagrams of PKR and E3L amino acid sequences to identify their similarities and differences, providing data for subsequent research.

3. Result

3.1. PKR and E3L Amino Acid Sequence Results

Edit the amino acid sequences of each PKR and E3L into the corresponding .TxT files, with the first line of each sequence starting with the symbol > followed immediately by the sequence name, and then enter the corresponding amino acid sequence on the next line (**Table 2**).

Table 2. Amino acid sequences corresponding to each PKR and E3L sequence name.

Sequence name	Amino acid sequence
>BvE3LdsRBM	PVTVINEYRQITRRDWSFRIESVGPSNSPTFYACVDIDGRVFDKADGKSKRDAKNNAAKLAVDKLL
>DvE3LdsRBM	PCSVLNEYCQYTSRDWYIDIISSGPIHKPLFTATLCISGVKFRSAIGSTKKEAKTNATRMAMDLII
>GvE3LdsRBM	PCSAINEYCQFTSRDWYINISSCGNGRKPMFLASVIISGIKFFPEIGNTKKEAKQKSTKRTIDFLI
>MvE3LdsRBM	PCTVLNEYCQITFREWSINVTRAGQSHSPTFTAVVTVSGYSFKSATGSNKKEARKNAAKEAMDVIL
>SwE3LdsRBM	PITVLNEYCQITQRDWIIDIISSGQSHCPIFTASITVSGIKCKTGKGSTKKEAKQIAARETMNFIL
>VvE3LdsRBM	PVTIINEYCQITKRDWSFRIESVGPSNSPTFYACVDIDGRVFDKADGKSKRDAKNNAAKLAVDKLL
>YvE3LdsRBM	PITVINEFCQITNRTAFYSIDSSGQSNNPIFYAYVTIDGRRFEMAEGKTKKEAKNKAAKNAVDKLF
>SvE3LdsRBM	PVSVLAEYCQHTRREWVFVEQQQGPLHSPTFVAYVTVSGSRFPVERAHTKKEARVAAARRAVEIIL
>DrPKRdsRBM1	YTSLLNEYQQKTQCTVEFEEGPTDGPSHNKRFTMRAIVNGQKFPDGTGKTKKEAKQNAAKNALEGLK
>DrPKRdsRBM2	YTCWLNEHSQKSRLMFKACESTKMDPGNLTRLCTYVCKYVCDDKEFPEGYGKNKKEAKEAAALQVYEELN
>DrPKRdsRBM3	YIAYLNNYCQKKKRVYDFKLVDRIGPPHNPIFVYKVVMDGKEYPEAQGRNAKEAKQNAAQHAWSEIR
>HsPKRdsRBM1	FMEELNTYRQKQGVVLKYQELPNSGPPHDRRFTFQVIIDGREFPEGEGRSKKEAKNAAAKLAVEILN
>HsPKRdsRBM2	YIGLINRIAQKKRLTVNYEQCASGVHGPEGFHYKCKMGQKEYSIGTGSTKQEAKQLAAKLAYLQIL

There are 13 dsRBM amino acid sequences from PKR and E3L used to construct a phylogenetic tree for comparative analysis, among which:

VvE3LdsRBM represents Variola virus E3L dsRBM (binding domain of the E3L protein of the smallpox virus)

GvE3LdsRBM represents Goatpox virus E3L dsRBM (binding domain of the E3L protein of the goatpox virus)

BvE3LdsRBM represents Buffalopox virus E3L dsRBM (binding domain of the E3L protein of the buffalopox virus)

MvE3LdsRBM represents Myxoma virus E3L dsRBM (binding domain of the E3L protein of the myxoma virus)

SvE3LdsRBM represents Squirrelpox virus E3L dsRBM (binding domain of the E3L protein of the squirrelpox virus)

YvE3LdsRBM represents Yokapox virus E3L dsRBM (binding domain of the E3L protein of the yokapox virus)

DvE3LdsRBM represents Deerpox virus E3L dsRBM (binding domain of the E3L protein of the deerpox virus)

SwE3LdsRBM represents Swinepox virus E3L dsRBM (binding domain of the E3L protein of the swinepox virus)

HsPKRdsRBM1 represents Homo sapiens PKR dsRBM1 (the first binding domain of human double-stranded RNA-activated protein kinase)

HsPKRdsRBM2 represents Homo sapiens PKR dsRBM2 (the second binding domain of human double-stranded RNA-activated protein kinase)

DrPKRdsRBM1 represents Danio rerio PKR dsRBM1 (the first binding domain of zebrafish double-stranded RNA-activated protein kinase)

DrPKRdsRBM2 represents Danio rerio PKR dsRBM2 (the second binding domain of zebrafish double-stranded RNA-activated protein kinase)

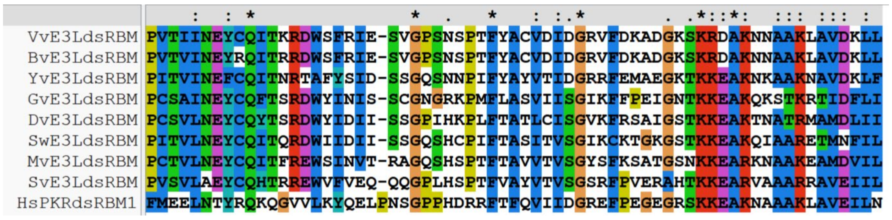
DrPKRdsRBM3 represents Danio rerio PKR dsRBM3 (the third binding domain of zebrafish double-stranded RNA-activated protein kinase).

3.2. Amino Acid Sequence Comparison Results of PKR and E3L

After preparing the amino acid sequences of PKR and E3L, use Clustal X2 software to perform sequence alignment on all sequences, set the output format to .aln, select complete alignment under the Alignment options, and after alignment, manually locate each nucleotide position.

3.2.1. Comparison of Amino Acid Sequences between Human PKR dsRBM1 and Viral E3L

A sequence comparison was performed between the dsRBM of the viral E3L protein and one dsRBM of human PKR. The length of the sequences involved in the comparison was 67 amino acid sequences, and 18 conserved sites were identified (including 14 highly conserved sites and 4 lowly conserved sites), accounting for 26.87% of the total positions. There were 6 conserved residues, representing 8.96% of the total positions (Figure 1).



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                :  : *              *  .   *   : :.*       . .*::*:  ::: ::: :
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 BvE3LdsRBM PVTVINEYRQITRRDWSFRIE-SVGPSNSPTFYACVDIDGRVFDKADGKSKRDAKNNAAKLAVDKLL
 YvE3LdsRBM PITVINEFCQITNRTAFYSID-SSGQSNNPIFYAYVTIDGRRFEMAEGKTKKEAKNKAAKNAVDKLF
 GvE3LdsRBM PCSAINEYCQFTSRDWYINIS-SCGNGRKPMFLASVIISGIKFFPEIGNTKKEAKQKSTKRTIDFLI
 DvE3LdsRBM PCSVLNEYCQYTSRDWYIDII-SSGPIHKPLFTATLCISGVKFRSAIGSTKKEAKTNATRMAMDLII
 SwE3LdsRBM PITVLNEYCQITQRDWIIDII-SSGQSHCPIFTASITVSGIKCKTGKGSTKKEAKQIAARETMNFIL
 MvE3LdsRBM PCTVLNEYCQITFREWSINVT-RAGQSHSPTFTAVVTVSGYSFKSATGSNKKEARKNAAKEAMDVIL
 SvE3LdsRBM PVSVLAEYCQHTRREWVFVEQ-QQGPLHSPTFVAYVTVSGSRFPVERAHTKKEARVAAARRAVEIIL
HsPKRdsRBM1 FMEELNTYRQKQGVVLKYQELPNSGPPHDRRFTFQVIIDGREFPEGEGRSKKEAKNAAAKLAVEILN
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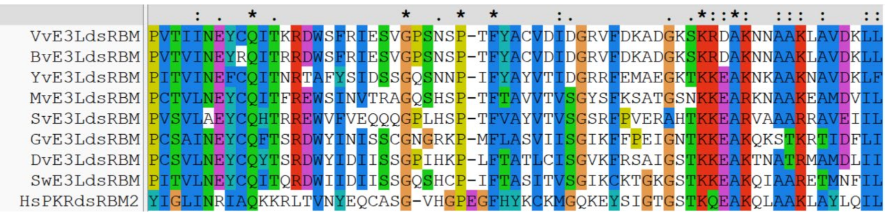
Figure 1. Alignment of the amino acid sequences of human PKR dsRBM1 and viral E3L.

3.2.2. Comparison of Amino Acid Sequences between Human PKR dsRBM2 and Viral E3L

A sequence alignment was performed between the dsRBM of the viral E3L protein and the two dsRBMs of human PKR. The sequences involved in the alignment were 67 amino acid sequences in length, revealing 17 conserved sites (11 highly conserved and 6 lowly conserved), accounting for 25.37% of the total positions. There were 6 conserved residues, representing 8.96% of the total positions (Figure 2).



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 VvE3LdsRBM PVTIINEYCQITKRDWSFRIESVGPSNSP-TFYACVDIDGRVFDKADGKSKRDAKNNAAKLAVDKLL
 BvE3LdsRBM PVTVINEYRQITRRDWSFRIESVGPSNSP-TFYACVDIDGRVFDKADGKSKRDAKNNAAKLAVDKLL
 YvE3LdsRBM PITVINEFCQITNRTAFYSIDSSGQSNNP-IFYAYVTIDGRRFEMAEGKTKKEAKNKAAKNAVDKLF
 MvE3LdsRBM PCTVLNEYCQITFREWSINVTRAGQSHSP-TFTAVVTVSGYSFKSATGSNKKEARKNAAKEAMDVIL
 SvE3LdsRBM PVSVLAEYCQHTRREWVFVEQQQGPLHSP-TFVAYVTVSGSRFPVERAHTKKEARVAAARRAVEIIL
 GvE3LdsRBM PCSAINEYCQFTSRDWYINISSCGNGRKP-MFLASVIISGIKFFPEIGNTKKEAKQKSTKRTIDFLI
 DvE3LdsRBM PCSVLNEYCQYTSRDWYIDIISSGPIHKP-LFTATLCISGVKFRSAIGSTKKEAKTNATRMAMDLII
 SwE3LdsRBM PITVLNEYCQITQRDWIIDIISSGQSHCP-IFTASITVSGIKCKTGKGSTKKEAKQIAARETMNFIL
HsPKRdsRBM2 YIGLINRIAQKKRLTVNYEQCASG-VHGPEGFHYKCKMGQKEYSIGTGSTKQEAKQLAAKLAYLQIL
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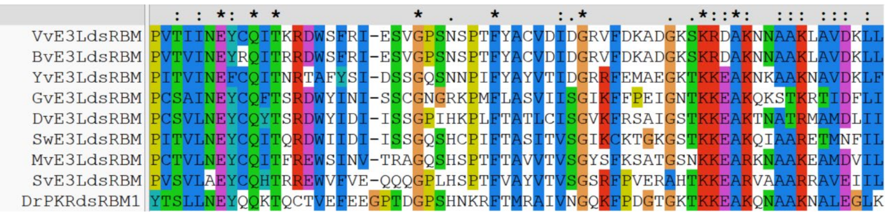
Figure 2. Alignment of the amino acid sequences of human PKR dsRBM2 and viral E3L.

3.2.3. Comparison of the Amino Acid Sequences of Zebrafish PKR dsRBM1 and Viral E3L

The dsRBM of the viral E3L protein was aligned with a dsRBM of zebrafish PKR. The sequences involved in the alignment were 67 amino acid sequences in length, revealing 18 conserved sites (14 highly conserved sites and 4 lowly conserved sites), accounting for 26.87% of the total positions. There were 8 conserved residues, representing 11.94% of the total positions (Figure 3).



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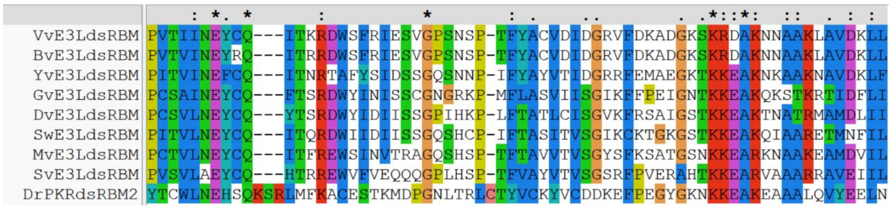
              : :*:  * *            *  .   *     :.*       . .*::*:  ::: ::: :
 VvE3LdsRBM PVTIINEYCQITKRDWSFRI-ESVGPSNSPTFYACVDIDGRVFDKADGKSKRDAKNNAAKLAVDKLL
 BvE3LdsRBM PVTVINEYRQITRRDWSFRI-ESVGPSNSPTFYACVDIDGRVFDKADGKSKRDAKNNAAKLAVDKLL
 YvE3LdsRBM PITVINEFCQITNRTAFYSI-DSSGQSNNPIFYAYVTIDGRRFEMAEGKTKKEAKNKAAKNAVDKLF
 GvE3LdsRBM PCSAINEYCQFTSRDWYINI-SSCGNGRKPMFLASVIISGIKFFPEIGNTKKEAKQKSTKRTIDFLI
 DvE3LdsRBM PCSVLNEYCQYTSRDWYIDI-ISSGPIHKPLFTATLCISGVKFRSAIGSTKKEAKTNATRMAMDLII
 SwE3LdsRBM PITVLNEYCQITQRDWIIDI-ISSGQSHCPIFTASITVSGIKCKTGKGSTKKEAKQIAARETMNFIL
 MvE3LdsRBM PCTVLNEYCQITFREWSINV-TRAGQSHSPTFTAVVTVSGYSFKSATGSNKKEARKNAAKEAMDVIL
 SvE3LdsRBM PVSVLAEYCQHTRREWVFVE-QQQGPLHSPTFVAYVTVSGSRFPVERAHTKKEARVAAARRAVEIIL
DrPKRdsRBM1 YTSLLNEYQQKTQCTVEFEEGPTDGPSHNKRFTMRAIVNGQKFPDGTGKTKKEAKQNAAKNALEGLK
```

Figure 3. Comparison of amino acid sequences between zebrafish PKR dsRBM1 and viral E3L.

3.2.4. Comparison of the Amino Acid Sequences of Zebrafish PKR dsRBM2 and Viral E3L

The dsRBM of the viral E3L protein was compared in sequence with the two dsRBMs of zebrafish PKR. The sequences involved in the comparison were 67 amino acid sequences in length, and 17 conserved sites were identified (including 10 highly conserved sites and 7 lowly conserved sites), accounting for 25.37% of the total positions. There were 5 conserved residues, accounting for 7.46% of the total positions (**Figure 4**).



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                : *. *      :         *       : .    ..       . .*::*:  ::  . : :
VvE3LdsRBM  PVTIINEYCQ---ITKRDWSFRIESVGPSNSP-TFYACVDIDGRVFDKADGKSKRDAKNNAAKLAVDKLL
BvE3LdsRBM  PVTVINEYRQ---ITRRDWSFRIESVGPSNSP-TFYACVDIDGRVFDKADGKSKRDAKNNAAKLAVDKLL
YvE3LdsRBM  PITVINEFCQ---ITNRTAFYSIDSSGQSNNP-IFYAYVTIDGRRFEMAEGKTKKEAKNKAAKNAVDKLF
GvE3LdsRBM  PCSAINEYCQ---FTSRDWYINISSCGNGRKP-MFLASVIISGIKFFPEIGNTKKEAKQKSTKRTIDFLI
DvE3LdsRBM  PCSVLNEYCQ---YTSRDWYIDIISSGPIHKP-LFTATLCISGVKFRSAIGSTKKEAKTNATRMAMDLII
SwE3LdsRBM  PITVLNEYCQ---ITQRDWIIDIISSGQSHCP-IFTASITVSGIKCKTGKGSTKKEAKQIAARETMNFIL
MvE3LdsRBM  PCTVLNEYCQ---ITFREWSINVTRAGQSHSP-TFTAVVTVSGYSFKSATGSNKKEARKNAAKEAMDVIL
SvE3LdsRBM  PVSVLAEYCQ---HTRREWVFVEQQQGPLHSP-TFVAYVTVSGSRFPVERAHTKKEARVAAARRAVEIIL
DrPKRdsRBM2 YTCWLNEHSQKSRLMFKACESTKMDPGNLTRLCTYVCKYVCDDKEFPEGYGKNKKEAKEAAALQVYEELN
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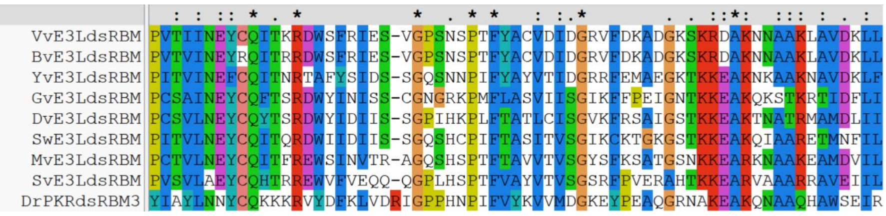
Figure 4. Amino acid sequence alignment of zebrafish PKR dsRBM2 and Viral E3L.

3.2.5. Comparison of the Amino Acid Sequences of Zebrafish PKR dsRBM3 and Viral E3L

The dsRBM of the viral E3L protein was aligned with the three dsRBMs of zebrafish PKR, with the aligned sequence length being 65 bp. A total of 20 conserved sites were identified (including 14 highly conserved sites and 6 lowly conserved sites), accounting for 30.77% of the total positions. There were 7 conserved residues, accounting for 10.77% of the total positions. This indicates that the dsRBM binding domain has high homology, high conservation, many conserved residues, and is relatively stable (**Figure 5**).



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VvE3LdsRBM  PVTIINEYCQITKRDWSFRIES-VGPSNSPTFYACVDIDGRVFDKADGKSKRDAKNNAAKLAVDKLL
BvE3LdsRBM  PVTVINEYRQITRRDWSFRIES-VGPSNSPTFYACVDIDGRVFDKADGKSKRDAKNNAAKLAVDKLL
YvE3LdsRBM  PITVINEFCQITNRTAFYSIDS-SGQSNNPIFYAYVTIDGRRFEMAEGKTKKEAKNKAAKNAVDKLF
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SwE3LdsRBM  PITVLNEYCQITQRDWIIDIIS-SGQSHCPIFTASITVSGIKCKTGKGSTKKEAKQIAARETMNFIL
MvE3LdsRBM  PCTVLNEYCQITFREWSINVTR-AGQSHSPTFTAVVTVSGYSFKSATGSNKKEARKNAAKEAMDVIL
SvE3LdsRBM  PVSVLAEYCQHTRREWVFVEQQ-QGPLHSPTFVAYVTVSGSRFPVERAHTKKEARVAAARRAVEIIL
DrPKRdsRBM3 YIAYLNNYCQKKKRVYDFKLVDRIGPPHNPIFVYKVVMDGKEYPEAQGRNAKEAKQNAAQHAWSEIR
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Figure 5. Comparison of the amino acid sequences of zebrafish PKR's dsRBM3 and viral E3L.

3.3. Results of Constructing the System Evolutionary Tree

According to the system phylogenetic tree (**Figure 6**), the evolution of the double-stranded RNA protein kinase PKR and the viral protein E3L's dsRBM system shows certain similarities, with a high evolutionary correlation between DrPKR and E3L. The tertiary structure of E3L interacts with PKR's dsRBM, inhibiting PKR phosphorylation. Studies have shown that the evolutionary origins of PKR and E3L have certain significance for viral prevention.

The system's evolutionary tree is as follows:

GvE3LdsRBM represents Goatpox virus E3L dsRBM (binding domain of Goatpox virus protein E3L)

DvE3LdsRBM represents Deerpox virus E3L dsRBM (binding domain of Deerpox virus protein E3L)

MvE3LdsRBM represents Myxoma virus E3L dsRBM (binding domain of Myxoma virus protein E3L)

SwE3LdsRBM represents Swinepox virus E3L dsRBM (binding domain of Swinepox virus protein E3L)

SvE3LdsRBM represents Squirrelpox virus E3L dsRBM (binding domain of Squirrelpox virus protein E3L)

YvE3LdsRBM represents Yokapox virus E3L dsRBM (binding domain of Yokapox virus protein E3L)

VvE3LdsRBM represents Variola virus E3L dsRBM (binding domain of Variola virus protein E3L)

BvE3LdsRBM represents Buffalopox virus E3L dsRBM (binding domain of Buffalopox virus protein E3L)

HsPKRdsRBM1 represents Homo sapiens PKR dsRBM1 (the first binding domain of human double-stranded RNA-activated protein kinase)

HsPKRdsRBM2 represents Homo sapiens PKR dsRBM2 (the second binding domain of human double-stranded RNA-activated protein kinase)

DrPKRdsRBM1 represents Danio rerio PKR dsRBM1 (the first binding domain of zebrafish double-stranded RNA-activated protein kinase)

DrPKRdsRBM2 represents Danio rerio PKR dsRBM2 (the second binding domain of zebrafish double-stranded RNA-activated protein kinase)

DrPKRdsRBM3 represents Danio rerio PKR dsRBM3 (the third binding domain of zebrafish double-stranded RNA-activated protein kinase).

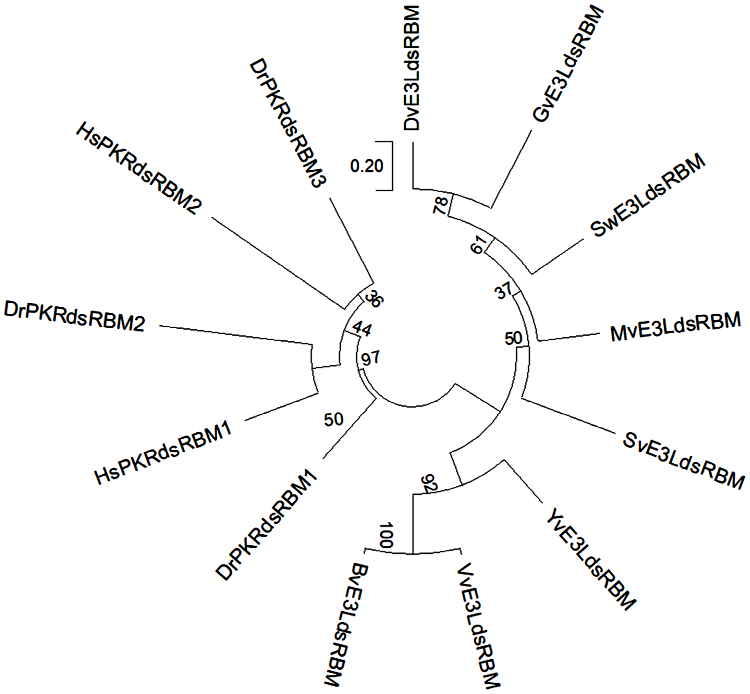

Figure 6. Structure of PKR and viral protein E3L and their evolutionary relationship in the system.

3.4. Molecular Docking Software and Scoring Algorithms Results

Through the results of molecular docking software and scoring algorithms (Figure 7), it was found that the dsRBM molecular spatial models of human PKR and the viral protein E3L both adopt an *α*-*β*-*β*-*β*-*α* fold, with highly similar structures.

Despite significant differences in amino acid sequences, their molecular structures are remarkably similar.

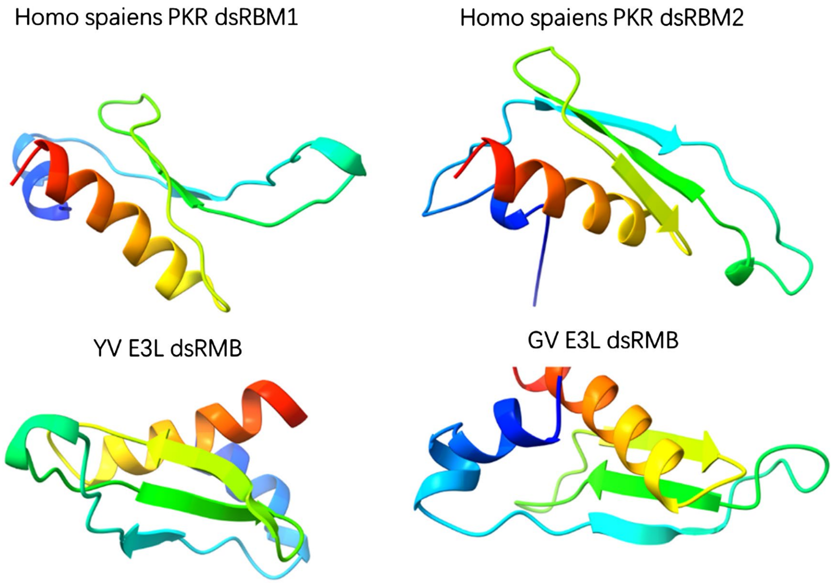

Figure 7. Molecular space simulation structure of viral protein E3L and human PKR dsRBM.

4. Discussion

Double-stranded RNA-dependent protein kinase (PKR) is an interferon-inducible protein involved in anti-cellular and antiviral activities. PKR inhibits translation initiation through the α subunit of the initiation factor eIF2 (eIF2α) and mediates various transcription factors such as NF-κB, p53, or STATs. Activated PKR can also induce apoptosis during vaccinia virus infection. Taking the human PKR protein structure as an example, it is composed of 551 amino acids in total, with the N-terminus containing two tandemly arranged dsRNA-binding motifs, dsRBM1 and dsRBM2, located in the 10-76aa and 101-166aa regions, respectively. Taking the zebrafish PKR protein structure as another example, it is composed of 682 amino acids in total, with the N-terminus containing three tandemly arranged dsRNA-binding motifs, dsRBM1, dsRBM2, and dsRBM3, located in the 8-74aa, 100-169aa, and 213-279aa regions, respectively. The regulatory domain at the N-terminus mainly mediates PKR's binding to dsRNA. DrPKR has the ability to bind dsRNA and autophosphorylate, and can phosphorylate zebrafish eIF2α in vitro, demonstrating that its function is homologous to mammalian PKR [10]. Fish such as zebrafish have two paralogous genes, PKR and PKZ, and their similarities and differences with human PKR in terms of structure, ligand recognition, and function [11]. PKR is one of the key molecules in the host antiviral pathway, and viral proteins can antagonize PKR's antiviral functions at different levels. These include: 1) binding to dsRNA to prevent dsRNA from binding to PKR; 2) expressing viral proteins to inhibit PKR dimerization; 3) producing pseudo-substrates of PKR; 4) activating phosphatases to dephosphorylate PKR; 5) inducing PKR degradation [12]. The RNA-binding protein E3 (E3L) is essential for viral interferon resistance, as it can directly bind to PKR to block its activation. Kim's study [13] determined the three-dimensional complex structure of E3L with PKR and identified key residues involved in the interaction. Additionally, PKR peptides can bind to E3L and, by upregulating phosphorylated PKR in HEK293 cells, increase the levels of cell apoptosis induced by phosphorylated PKR and phosphorylated eIF2α. The study found [13] that PKR peptides bind to E3L more effectively than PKR protein, blocking E3L function and can be used as potent inhibitors of the variola virus. It has been reported [14] that the direct interaction between E3L and PKR, in which the C-terminal region of E3L can downregulate the spontaneous activation of PKR and the subsequent phosphorylation of eIF2. Although the E3L protein is a double-stranded RNA-binding protein and can sequester double-stranded RNA that activates cellular proteins, this mechanism seems to require selective RNA structures or cellular localization to enable potential target selection beyond PKR. The study uses a transgenic zebrafish embryo infection model to screen a large compound library for lead compounds that can inhibit the stability of the Zika virus capsid protein, which are then applied to the screening of drugs that modulate the PKR pathway [15]. Studies have shown [16] that ADAR1 can inhibit PKR, and this inhibition is independent of RNA editing but requires dsRBD3. According to the results shown in Figures 1-5, viral E3L has high conservation with the third dsRBM of zebrafish, with many conserved residues, suggesting that E3L heterodimerizes with the third dsRBM of zebrafish PKR. The conserved amino acids that determine this heterodimerization are mainly concentrated at positions 3, 5, 7, 8, 35, 37, 50, 51, 53, 56, 57, 58, 60, and 64. It has been reported [17] that the N-terminal ZBD domain and the C-terminal dsRNA-binding domain of the E3L protein both have the function of inhibiting apoptosis. The E3L-PKR structural system was studied and analyzed through bioinformatics. Since E3L can mimic the structure of PKR and interact with PKR, research on PKR and E3L provides a foundation for understanding the antiviral mechanisms of PKR (such as against vaccinia virus, adenovirus, influenza virus, etc.), aiding the design and development of novel antiviral drugs or providing therapeutic targets, and having profound significance for effective prevention and control. Molecular docking software and scoring algorithms also indicates that the three-dimensional structure of E3L-PKR and their interactions could help in designing new antiviral therapeutic targets.

However, a limitation of this study is that the sample types are relatively few, which cannot comprehensively reflect the relationship between the viral protein E3L and various biological PKRs. Therefore, further research through animal experiments and cell experiments is needed to study the mechanism by which PKR and the viral protein E3L inhibit phosphorylation, their molecular structure, and mode of action. This will help regulate the virus replication mechanism in host tissues, which has very important practical significance for viral immune prevention, and further provides new ideas for the development of antiviral drugs.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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