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The in silico interaction analysis of CARMIL1 protein-containing leucine-rich repeat (LRR) regions with interleukin-1 receptor-associated kinase 1 (IRAK1) protein and LLR peptide

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ABSTRACT

Objectives: Capping protein Arp2/3 and myosin-I linker protein 1 (CARMIL1) encoded by the CARMIL, is a major, multidomain, membrane-linked protein regulating actin assembly; however, its function in inflammatory signaling is not fully elucidated. The leucine-rich repeat (LRR) region of CARMIL1 has been associated with interleukin (IL)-1 receptor-associated kinase (IRAK) in fibroblasts by many methods including tandem mass tag mass spectrometry, immunoprecipitation, and CRISPR-Cas9. This study, therefore, set out to assess the interaction of CARMIL1 with each IRAK1 protein and a novel LRR peptide.

Methods: The molecular docking techniques were employed to compare the binding modes and affinities of the 3D structure of CARMIL1 each of LRR peptides and IRAK1 protein. 3D structure model of CARMIL1 protein and LRR peptide was predicted through Robetta tool considering the structures and function of these proteins.

Results: As an overall conclusion of docking, the LRR peptide was observed to contact the residues in the LRR 1-2 of the human CARMIL1, whereas the IRAK1 protein was to interact with the residues in the LRR 1, 2, and 10 regions of the human CARMIL1.

Conclusions: Our computational results suggest that LRRs in CARMIL1 are involved in the formation of protein-peptide binding interfaces with its structural conformation.

Keywords: CARMIL1, IRAK1, LRR peptide, molecular docking simulation

Interleukin-1 (IL-1), a critical intermediate for inflammation, assists the degradation and remodeling of extracellular matrices (ECMs) by enhancing the expression of matrix metalloproteinases (MMPs), thus, plays a central role in various inflammatory diseases including rheumatoid arthritis, chronic periodontitis, and severe lung injury [1]. The binding of IL-1 to its signal receptor [IL-1 receptor type 1 (IL-1R1)], allows the IL1R assistant proteins to be recruited. Activation of the IL-1R complex is followed by recruitment of MyD88 (myeloid differentiation factor 88). This newly formed adapter protein complex then binds to IRAK1 and IRAK2 (Interleukin-1 receptor-associated kinase 1 and 2) [2]. IRAK is quickly phosphorylated, subsequently released from MyD88, and initiates signal transduction pathways, like the mitogen-activated

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protein kinase (MAPK) family members ERK [3].

Fibroblasts are a major group of cells in the connective tissues, directly included in the matrix degradation in widespread inflammatory diseases such as periodontitis and rheumatoid arthritis. Concerning IL-1 signal transmission in fibroblasts, IL-1R1 needs to be recruited to focal adhesions (Fas) [4]. Following this recruitment, FA kinase is activated, Ca2+ is released from the endoplasmic reticulum [5], and ERK is activated eventually [3]. Such mechanisms in the central parts of the signaling system increase the level of MMPs expression [6]. Despite the comprehensive examinations, the control of IL-1 signaling in anchorage-dependent stromal cells is not fully elucidated.

Human CARMIL1 (Capping Protein Arp2/3 myosin I linker) from CARMIL-family is a large multidomain protein, profoundly conserved. CARMIL proteins have several membrane-associated functions associated with actin assembly and signaling owing to their structural characteristics. CARMIL1 is expressed in plenty by fibroblasts, which is intensely linked to abnormal inflammatory processes [7].

CARMIL1 is approximately 1370-aa length protein with 16 leucine-rich repeats (LRRs), which has a non-canonical pleckstrin homology (PH) domain, a long helical domain (HD), and a C-terminus responsible for the interaction with F-actin-capping protein subunit alpha-2 (CAPZA2) [8, 9].

In a recent study, Wang *et al*. [10] have investigated the function of CARMIL1, its LRR region, and CPI-CSI motifs (CP-binding domain) in controlling the production of IL-1 signaling in circulating fibroblasts. They have concluded that the LRR of CARMIL1 would mediate IL-1-induced collagen degradation in the fibroblasts and might be a goal for the anti-inflammatory drug improvement. In the current study, inspired by the experimental findings from this recent investigation, we computed the interaction of LLR regions in a model of human CARMIL1 protein with IRAK1 protein and the designed LRR peptide by drawing on the tested/trusted bioinformatics tools and databases to find the polar contacts. The cellular and biological role of a protein is broadly direct linked to its 3D structure [11]. In this context, since Carmil1 contains the leucine-rich repeat as a protein recognition motif whose structural characterization of this

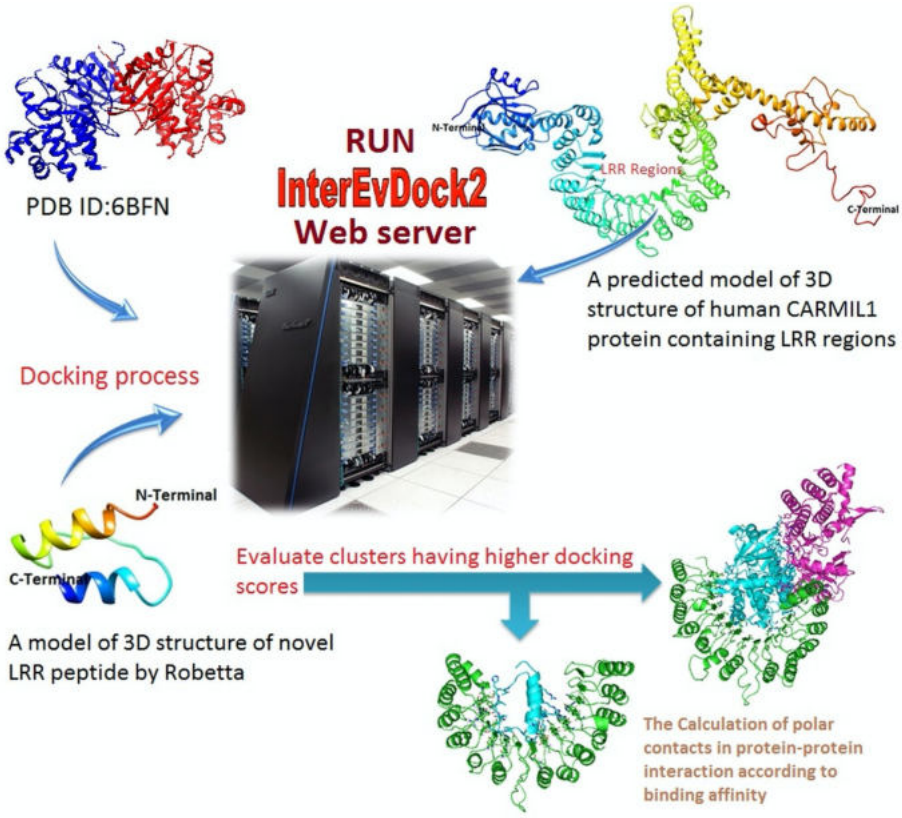

Fig. 1. A shortened work-schema of molecular docking of CARMIL1 with each of IRAK1 and LRR peptide.

motif has been implicated as a critical for the development of targeted drugs. Hence, the three-dimensional structure of the CARMIL1 protein and its conformation and binding interfaces at the atomic level with the novel LRR peptide was investigated. Through these findings, we examined what molecular features of LRR regions are responsible for interaction with the IRAK1 (Fig. 1).

METHODS

The Obtaining of the 3D Structure of Protein and Peptide

First, the protein sequence encoded by human CARMIL1 (UniProtKB/Swiss-Prot: Q5VZK9.1) in FASTA format was obtained from NCBI. LRR peptide sequence (GRKKRRQRRRPQTLVHLDLSGNVLRGDDLSHMYNFLAQPNK) was fetched from the paper of Wang *et al*. [10]. Then, each of the amino acid sequences were then subjected to the Robetta [12, 13], a common tool for homology-based approaches to possess a three-dimensional structure of CARMIL1 and LRR peptide as a model (see Fig. 2). The crystal structure of human IRAK1 (PDB ID: 6BFN) from PDB (Protein Data Bank) at http://www.rcsb.org/ was downloaded in PDB format. In Rosetta server job submission, it was used comparative modeling and all settings left as default and generated five 3D-structure models. It was selected the most accurate one from model proteins according to confidence scores, which indicates the accuracy of model protein in terms of predicted GDT (1.0 good, 0.0 bad).

Pre-Preparation for Molecular Docking

Energy Minimization and Quality Control of Model Protein Structures

The energy minimization of 3D model protein structures was subjected to the minimization method in chimera 1.14 [13]. The default steepest descent was set to 100 with 0.02 step sizes, without fixing any atoms, and was followed by 10 steps of conjugate gradient steps with 0.02 step size (Å) minimization. To control the quality of the model proteins, we evaluated both the structural quality using Qualitative Model Energy Analysis (QMEANDisCo) [15], as well as Ramachandran plots that were drawn to assign key secondary structures to specific regions in the plot.

Sequence Analysis

The protein sequence encoded by Human CARMIL1 (Reference Sequence: NP_060110.4) was retrieved from NCBI in FASTA format and run via PSI-BLAST (Position-Specific Iterated BLAST) [16]. As search setting, it was selected Protein Data Bank (PDB) proteins, as all general algorithm parameters including MATRIX: BLOSUM62, Existence:11 Extension:1, the threshold value (0.05), world size: 6, and

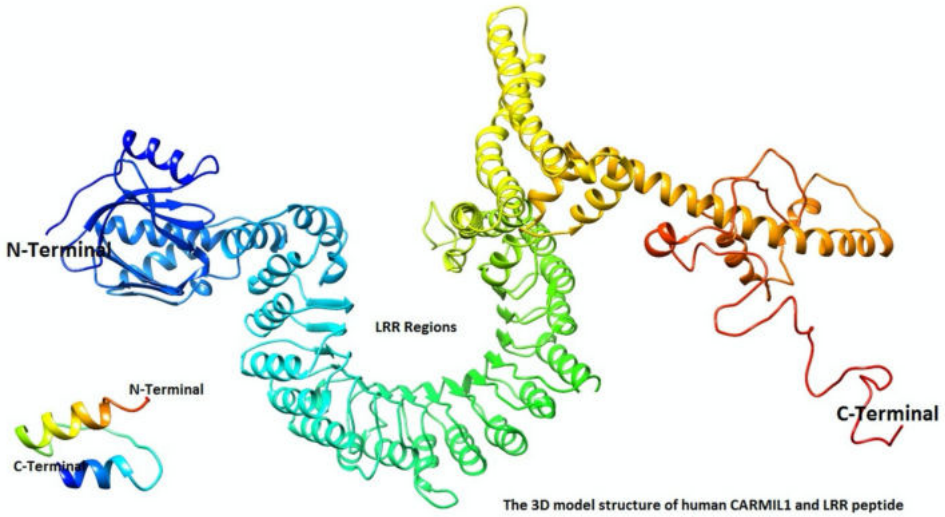

Fig. 2. The 3D structure of predicted CARMIL1 and LRR peptide. Robetta server predicted the 3D model structure of CARMIL1 protein (1-1000aa) and LRR peptide (1-41aa) by the homology-based model. The model was subjected to a mode of protein-protein and peptide docking. The cartoon model representation and image were produced with Chimera 1.14. Structures are symbolized as interactive colored ribbons to view the strand and helix forms.

Compositional adjustments: conditional compositional score matrix were left as the default settings.

Visualization of Molecular Docking Simulations

In this study, it was employed the method that one of the computer-aided drug design (CADD) approaches is structure-based drug design (SBDD) [17]. SBDD methods analyze macromolecular targeting 3D structural details, commonly of proteins or RNAs, to determine fundamental parts and interactions that are significant for their regarding biological procedures. In the docking process, there is no restrictions were placed between surface-exposed residues of protein-peptide and protein-protein but conversely performed blind docking. In InterEvDock2 docking server, as demonstration Mode for docking procedure, standard usage (easy level) was opted. Two protein sequences or structures and their respective multiple sequence alignments are employed to estimated binding modes via a free docking process at the web server.

The primary structure of CARMIL1 was colored by drawing on Jalview 2.11.1.3 [18]. PyMOL software [19] was used to illustrate the tertiary structure proteins-peptides and analyze the molecular modeling re-

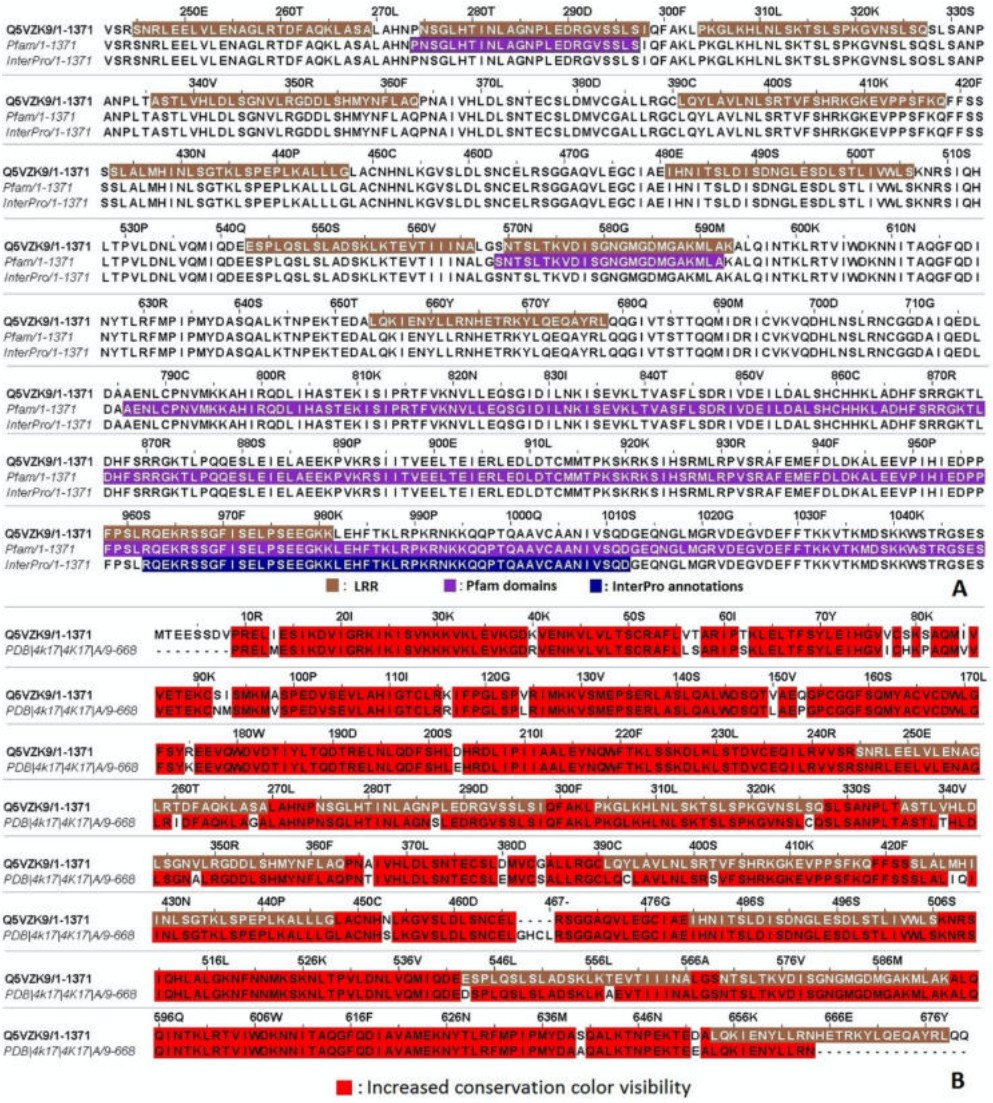

Fig. 3. (A) The visualization of the Pfam domain (purple), InterPro annotations (navy blue), and LRR regions (brown) in the CARMIL1 aa sequences. LRR_6 (274-297aa, 569-592aa), CARMIL_C (786-1081aa) from Pfam domains, Leucine-rich repeat-containing protein 16A (962-1012aa) from InterPro domains, There are 11 parts of known LRR regions from Uniprot. All feature sequences were retrieved from Uniprot and PDBeKB. (B) Representation of the primary structure of Human CARMIL1 protein with Mouse CARMIL1 in similar sequences after alignment. This figure solely provides the LRR region sequences in the human CARMIL1.

sults at the 3D atomic level. All complexes of protein-protein interactions were performed by a fully automatized InterEvDock2 docking server [20-22] through utilizing the SOAP_PP [23] and FRODOCK2 [24]. In the docking process, there is restrictions were placed between surface-exposed residues of protein-peptide and protein-protein but conversely performed blind docking. In InterEvDock2 docking server, as demonstration Mode for docking procedure, standard usage (easy level) was opted. Two protein sequences or structures and their respective multiple sequence alignments are employed to estimated binding modes via a free docking process at the web server.

RESULTS

The Alignments and Sequence Analysis

According to alignment results obtained through the PSI-BLAST tool, the CARMIL1 sequence demonstrated a 91.45% per identity and a query cover 48% with Chain A, Leucine-rich Repeat-containing Protein 16a [Mus musculus Accession: 4K17_A] as a top homolog protein. Thus, this protein was selected as a template for the homology modeling by the Robetta server. Following the alignments of the sequence of CARMIL1 and the template protein, we detected similar sequences in LRR regions (Fig. 3, section B). This finding boosts the accuracy of the prediction of the three-dimensional structure of the LRR regions of the CARMIL model protein.

Docking Consequences of CARMIL1 with Each of LRR Peptide and IRAK1

The docking scores and the residues of polar contacts between CARMIL1 and LRR peptides are listed in Table 1-2. As can be seen from Table 1, the top 10 consensus complexes include IES1 and FRODOCK2 with higher energy scores and SOAP_PP4 with lower energy scores. According to the online prediction tools FRODOCK2 and InterEvDock2, in all top docking complexes, E254 (the LRR1 in CARMIL1), K3, and R2 (LRR peptide), which are the top 5 residues (on each chain) on the consensus of the top 10 models, were predicted to be involved in contacts (see Fig. 4 sec. 3.1, and 4.1, Fig. 5 sec. 7.1 and 10.1). As an overall conclusion of docking, the LRR peptide contacted the residues in the LRR regions (one, seven) of the human CARMIL1.

FRODOCK1 and IES1, and SOAP_PP1 are the top consensus complexes by higher docking scores within the representative models from the 10 best clusters (see Table 2). According to the online prediction tools FRODOCK2 and InterEvDock2, in all top dock-

Table 1. The results obtained from the analysis of the docking CARMIL1 and LRR peptide

Docking complexes	LRR peptide	CARMIL1 (Residue number)	LRR region	Docking score
SOAP_PP9	R5, S20, S30, Q38	**H428**, **S432**, G456, **T485**, R630	LRR6, 7	-16724.74
FRODOCK6	R25	**D488**, **D577**	LRR7, 9	1909.43
SOAP_PP10	R2, R6, R10, K41	**E254**, **S313**, **S345**, D460, N463, **S490**, **S550**	LRR1, 3, 4, 7	-16539.82
FRODOCK7	K3, R25	**E254**, **D343**	LRR1, 4	1888.22
IES1	Q7, R8, H16	E475, G476, **T500**	LRR7	33.71
IES3	Y33, G26, D27	I478, **D497**, **S499**	LRR7	31.7
SOAP_PP1	R2, H31, N40	**E254**, **N255**, **N311**, **S313**, D371, **D488**	LRR1, 3, 7	-16991.95
SOAP_PP2	K41, S30	**E254**, **K265**	LRR1	-16925.85
FRODOCK2	R6	**E254**, D460, K608	LRR1	2060.55
SOAP_PP4	K3, R10, Y33, P39, N40	**E254**, **N255**, S373, **S490**, **K575**	LRR1, 7, 9	-16773.15

The table provides the residues of polar contacts between the model CARMIL1 and LRR peptide. The residue numbers of LRR regions in CARMIL1 are shown in bold font. The lower score is better in the docked complexes of SOAP_PP whilst the higher score is better in the docked complexes of FRODOCK and IES

Table 2. The results obtained from the analysis of the docking CARMIL1 and IRAK1 are presented.

Docking complexes	IRAK1	CARMIL1 (Residue number)	LRR region	Docking score
1.FRODOCK5	K242, N211	**E249**, **E254**, R630	LRR1	2335.05
2.IES1	Q254, T258, Y277, R194	**V252, A256, D261, E289**	LRR1, 2	820.97
3.IES2	Q254, Y277	**E250, R259**	LRR1	697.85
4.IES3	T383, T431	**E254, D261**	LRR1	658.16
5.IES4	R363, Q254, N211	**E254, N255, Q655**	LRR1,10	635.63
6.SOAP_PP1	S331, R194	K644, **E651**, **E658**	LRR10	-34824.7
7.SOAP_PP2	R194, K253, S333, S335	**N255**, N374, Y637, S640	LRR1	-34575.19
8.SOAP_PP3	Y277, S193, R366	**A256**, **G257**, Y637	LRR1	-34535.14
9.FRODOCK1	E248, S333	**T280**, **N282**, R630	LRR2	2548.03
10.FRODOCK2	R366, K253, Y284	**T280**, H452, **L661**, **L662**, **R663**	LRR2, 10	2431.33

The table provides the residues of polar contacts between the model CARMIL1 and IRAK1 protein. The residue numbers of LRR regions in CARMIL1 are shown in bont font. The lower score is better in the docked complexes of SOAP_PP whilst the higher score is better in the docked complexes of FRODOCK and IES.

ing complexes, E250, V252, E254 (the LRR1 in CARMIL1) and R194, Q254, Y277 (IRAK1 protein), which are the top 5 residues (on each chain) on the consensus of the top 10 models, were predicted to be involved in contacts (see Fig. 6 sec. 2.1, 3.1, 3.2, and 5.1). As a general result of the docking process, IRAK1 protein contacted the residues in the LRR regions (one, two, and ten) of the human CARMIL1.

DISCUSSION

This study explores to address the in silico analysis of human CARMIL1 protein, which recently was associated with IL-1-induced ERK activation and MPPs expression and whose 3D structure is not fully uncovered yet. CARMIL1, which has an N-terminal domain-containing LRRs that provide broad contacts with other regulatory proteins, is an actin regulator with diverse functions [7]. Until recently, there has been no reliable evidence concerning the role of CARMIL1 in inflammation through the degradation of ECMs by elevating the expression of MMPs. Given the strong molecular evidence, this protein assures a therapeutic target associated with inflammatory diseases such as rheumatoid arthritis, acute lung injury, and chronic periodontitis [10]. However, much uncertainty still exists about the relation between CARMIL1 and inflammatory disorders regarding targeted therapeutic drug development.

In this study, we aimed to predict the model interactions of CARMIL1 with the IRAK1 that displays a functional feature through interacting with CARMIL1 for ERK activation and MMPs expression, and with a novel LRR peptide.

Robetta is a protein structure prediction service continuously evaluated with CAMEO (Continuous Automated Model Evaluation), which constantly assesses the accuracy and reliability of the prediction. The other prediction tools including CAMEO, Robetta, and QMEAN are among the first-line by time-based statistical confidence and reliable performances. The InterEvScore used in the study has determined the heteromeric protein interfaces (polar contacts) and the integration of the evolutionary information obtained from the multiple sequence alignments of each protein in the clusters with a residual-based multi-body statistical potential. In this prediction server, docking searching is systematically implemented utilizing the FRODOCK2 and the outcomes are re-calculated by InterEvScore [22] and SOAP_PP atom-based statistical potential to enhance the confidence level of the

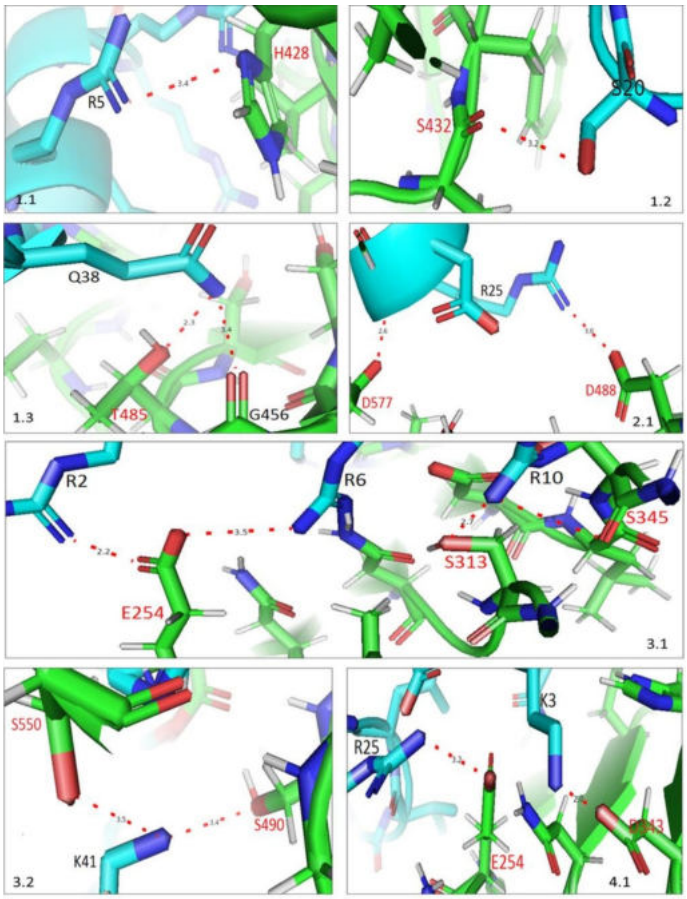

Fig. 4. The InterEvDock2, SOAP_PP, and FRODOCK2 model results of CARMIL1 protein and LRR peptide. The docking complexes are represented in a protein interface representation by PyMOL, colored by (CARMIL1 in green, its interaction residues font in LRR regions in red, and LRR peptide in cyan, and possible polar contacts (dash lines) in red). The sections in the figures are enumerated in the order of the list in Table 1 (Such 1.1-4.1). Only figures from docking pose related to LRR regions are concerned.

predictions.

The structural details present in the predicted protein model are significant for defining the biological function of the modeled protein and its use in future experiments, model verification has a critical step in protein structure prediction. To assess the quality level of secondary structure of model CARMIL1 protein and LRR peptide, we drew the Ramachandran plots through the webserver at https://swissmodel.expasy.org. The Ramachandran plots are represented as an x–y plot of the Phi (φ) and Psi (ψ) of the dihedral angles between N-Ca and Ca-C planar peptide bonds in a protein's backbone. The Phi (φ) and Psi (ψ) are regarded as conformational angles that determine the conformation of the whole chain of a protein. All theoretically probable secondary structures are shaded in orange and dark blue as the most favored regions in the plots (Figs. 8 and 9). To interpret these plots, we used the outputs of MolProbity [25] tool, which is a structure-validation web service that provides evaluation of model quality. Ramachandran Favoured score is 96.66% for the CARMIL1, while this score is 100.00 % for LRR peptide. The Ideal score for the Ramachandran Favoured in the MolProbity tool has been reported > 98% as an ideal case.

Protein structures can contain multiple intense foldable parts, namely domains. These domains comprise typical hydrophobic cores, and can be folded free of each other, and are nearly always connected to establish diverse roles [26]. In this manner, our first pre-

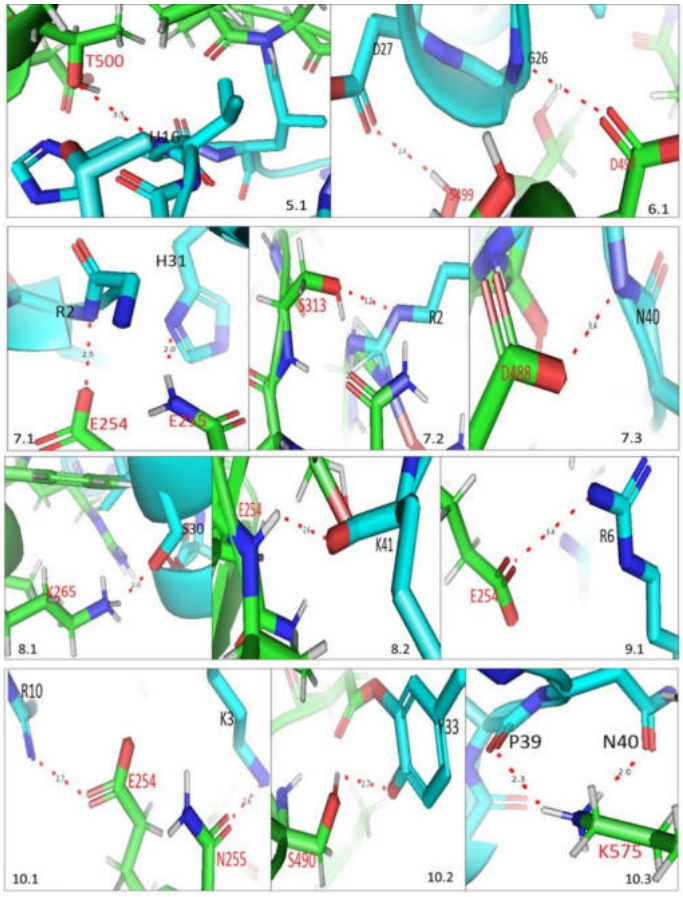

Fig. 5. The docking complexes are represented in a protein interface representation by PyMOL, colored by (CARMIL1 in green, its interaction residues font in LRR regions in red, and LRR peptide in cyan, and possible polar contacts (dash lines) in red). The sections in the figures are enumerated in the order of the list in Table 1 (Such 5.1-10.3). Only figures from docking pose related to LRR regions are concerned.

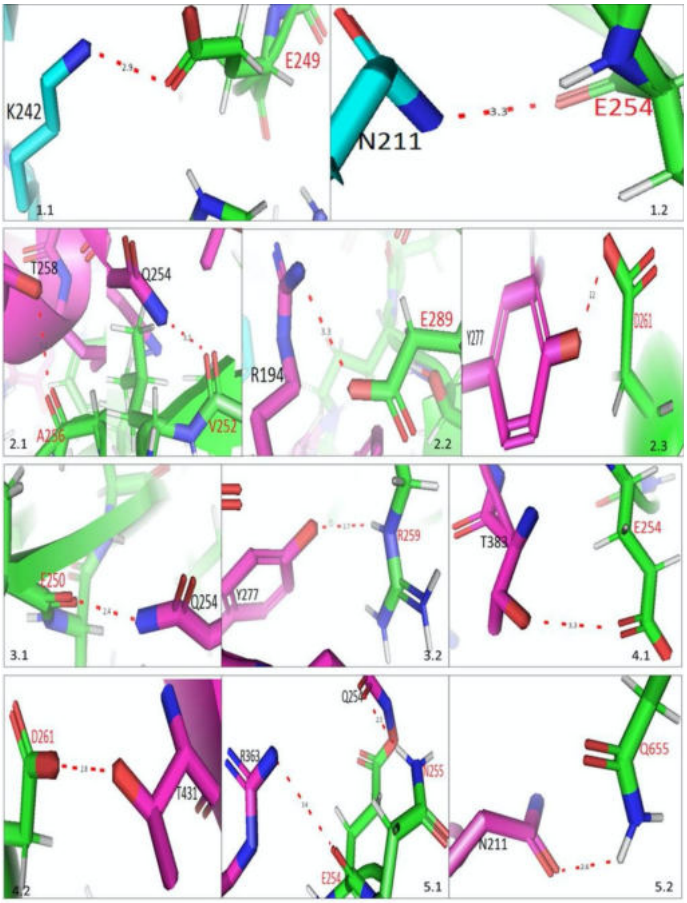

Fig. 6. The InterEvDock2, SOAP_PP, and FRODOCK2 model results of the CARMIL1 and IRAK1 protein. Binding modes of the CARMIL1 and IRAK1 in the protein interface representations are shown, colored by (CARMIL1 in green, its interaction residues font in LRR regions in red, and IRAK1 protein in cyan and magenta). The sections in the figures are enumerated in the order of the list in Table 2. (Such 1.1-5.2) Only figures from docking pose related to LRR regions are included.

liminary approach in this study was to analyze the LRR regions in CARMIL1 through Uniprot and PDBe-knowledge base (see Fig. 3, section A) since Wang *et al*. [6] demonstrated that the LRRs of CARMIL1 associate with IL-1 signaling proteins.

LRRs are nearly 21-28 residues (Figs. 6 and 7).s sequence motifs existing in various proteins with distinct functions. The main role of these motifs seems to implement a multidirectional structural framework for the composition of protein-protein interactions. Recent structural information in the LRR proteins has heightened the need for our opinion concerning the structural factors, our experience to model such proteins with uncharacterized structures, and has illuminated how these proteins attend in protein-protein interactions [27]. For this purpose, the molecular docking process may be a satisfactory and robust computational approach to recognize the function of LRRs in protein-protein interaction at the atomic level.

The cell-permeable, CARMIL1 binding LRR peptide, has been reported to inhibit IL-1-induced collagen degradation by MMPs [6]. The novel LRR peptide consists of 41 amino acids, most of which are basic and hydrophilic (see part 2.1.). The results, as shown in Figs. 4 and 5, indicate that lysine(K) and arginine(R) in the LRR peptide are the basic amino acids, whereas glutamate (E) and aspartate(D) in the CARMIL1 are acidic amino acids in the most parts of whose interaction poses in the complexes. Besides, according to the calculation results, the LRR peptide mostly interacts with the residues in the LRR 1 and LRR 7 regions (see Table 1).

Binding modes of CARMIL1 with the IRAK1 protein by molecular docking simulation are displayed in Figs. 3 and 4. In general, IRAK1 interacted with the protein kinase domain between the residue number of 212-521 (see Table 2). As a computational output, residues Q254 and Y277 in the protein kinase domain are the most common polar interactions with CARMIL1, while CARMIL1 binds to IRAK1 through

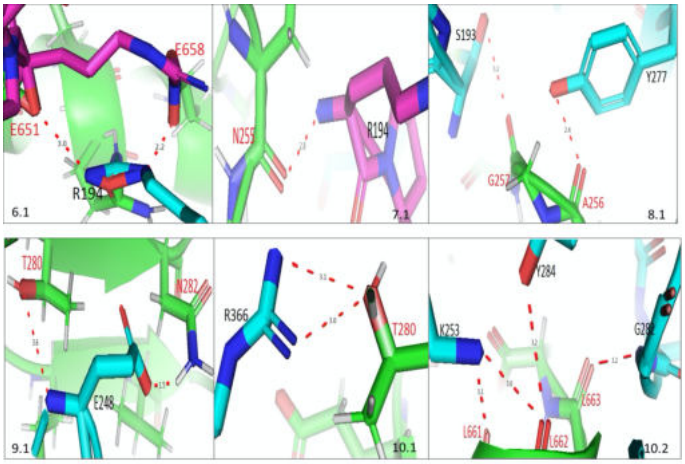

Fig. 7. The InterEvDock2, SOAP_PP, and FRODOCK2 model results of the CARMIL1 and IRAK1 protein. Binding modes of the CARMIL1 and IRAK1 in the protein interface representations are shown, colored by (CARMIL1 in green, its interaction residues font in LRR regions in red, and IRAK1 protein in cyan and magenta). The sections in the figures are enumerated in the order of the list in Table 2. (Such 6.1-10.2) Only figures from docking pose related to LRR regions are included.

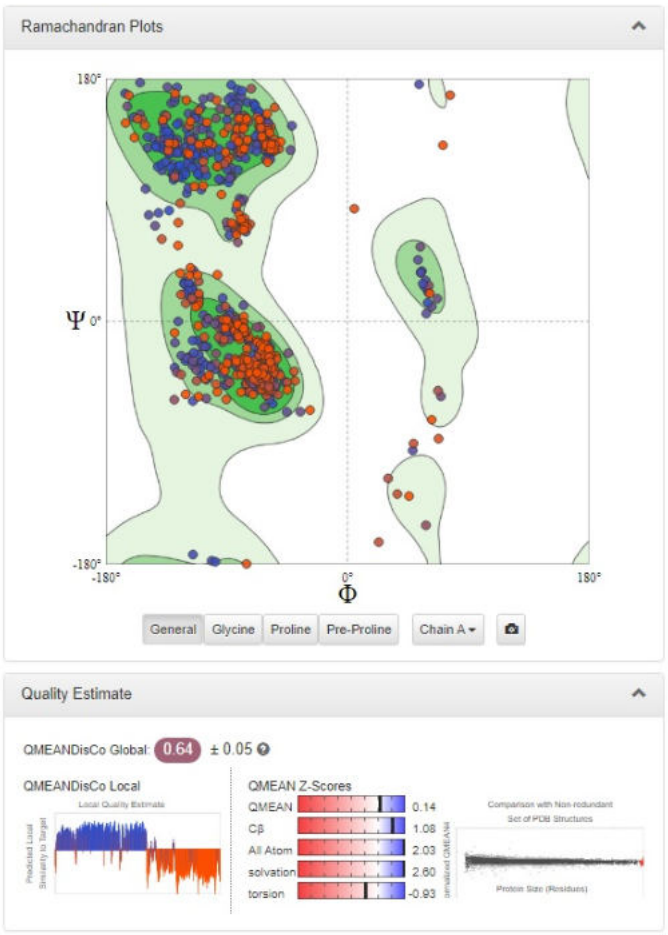

Fig. 8. Ramachandrant and structure quality estimate plot of CARMIL1 from the webserver at https://swissmodel.expasy.org.

Fig. 9. Ramachandrant and structure quality estimate plot of LRR peptide from the webserver at https://swissmodel.expasy.org.

V252, E254 residues in LLR1 (see Figs. 6 and 7). According to the results of both the LRR peptide and IRAK1 protein docking with the model of human CARMIL1 protein, E254, N254, and R630 commonly interacted residues. All docked complexes with the LRR peptide and IRAK1 are related to LRRs regions in CARMIL1. These calculations would seem to suggest that the primary function of the LRRs performs a sophisticated structural frame for the formation of protein-protein interactions.

Limitations

As limitations of the study, to be sure and explain the stability of the molecular docking complexes, classical molecular dynamic (MD) process is advised to conduct. InterEvDock2 docking server is presently not able to dock nucleic acids or small molecules. When nucleic acids or ligands are present in a protein chain, they will be kept only as steric objects. In this study, as only one ligand is conducted, comparison assessment is recommended by targeting a similar ligand to the LRR region of the CARMIL1 protein just as the LRR peptide is targeted.

CONCLUSION

Inflammation is normally a response of the body to infection and ailment. However, it may be seldomly misled, hereby the immune system preferably attacks healthy tissues. In the current study, an in-silico analysis was performed to predict the polar contacts to evaluate the binding mode and affinities of the CARMIL1 with the IRAK1 protein and the novel LRR peptide. The docking score and protein-protein interaction with significant amino acid residues were identified as hits

through the docking server. Both the LRR peptide and IRAK1 protein contacted with the residues of E254 and N255 in the LRR1 region of human CARMIL1. The outcomes indicate that the LRR1 region is more significant in evaluating the LRR peptide for its effectiveness in inhibiting the interaction of IRAK1 and CARMIL1 protein. Taken together, as three scoring programs in the docking process have confirmed each other, we conclude that the models with the highest binding energy are the complexes that interact with residues in the LRRs regions of the CARMIL1. This study may contribute to future studies, as CARMIL1 being a promising target for anti-inflammatory drug development.

Authors' Contribution

Study Conception: NB, GY; Study Design: NB, GY; Supervision: NB, GY; Funding: NB, GY; Materials: NB, GY; Data Collection and/or Processing: NB, GY; Statistical Analysis and/or Data Interpretation: NB, GY; Literature Review: NB, GY; Manuscript Preparation: NB, GY and Critical Review: NB, GY.

Ethical approval

There are no studies with the human participant or animal performed by any of the authors in this paper.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

Financing

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