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Computational molecular characterization of p53 and Human TLRs interactions

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ABSTRACT

Toll-like receptors (TLRs) are one of the major sensors to regulate innate immunity. It is present in inactive form within immune cells. However, after recognizing the conserved region of the foreign body, it gets activated by the foreign body, such as bacteria, viruses, fungus, etc. Recently, it is reported that apart from participating in innate immunity, these TLRs also play an important role in apoptosis and cancer. Moreover, very few reported that it is cross-talk with p53 protein within the cell. P53 protein is a transcription factor for many cellular proteins involved in cellular transduction. It directly as well as indirectly regulates a wide variety of cellular processes such as apoptosis, senescence, cell cycle arrest, differentiation, and DNA repair and replication and cancer dynamics. Various studies reported genetic level interaction between p53 and TLRs. However, molecular interaction studies are still few reported. In the present work, we computationally characterized molecular interaction between p53 and toll-like receptors. We used open web resources for docking and analyzing the data. Our molecular docking and molecular dynamics simulation results suggest that there is a significant interaction between p53 and toll-like receptors. The study could important for the possible therapeutic intervention.

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Introduction

TLRs proteins are major sensor molecules within the immune cells. Immune cells, such as macrophages, dendritic cells, mast cells, neutrophils, epithelial cells, are considered as the first line of defense against the invading bacterial, viral and fungal infections (1-3) each of the immune cells collectively coordinated with each other to act against the foreign body. Moreover, TLRs are specialized receptor proteins found in immune cells. TLRs proteins belong to a group of receptor proteins generally known as PRRs (pattern recognition receptors) (4-6). In addition, these PRRs are specialized due to their recognition of conserved patterns of molecular structures of the pathogen, known as PAMP (pathogen-associated molecular pattern). TLRs are generally present in the inactive state within the immune cells. However, after recognition of the conserved region of the foreign body, it gets activated. The activation of TLRs leads to the activation of various other proteins responsible for the production of interferons. Recently, it is reported that apart from participating in innate immunity, these TLRs also play an important role in apoptosis and cancer. Moreover, very few reported that it is cross-talk with p53 protein within the cell.

P53 is one of the major proteins within the cells, which is widely studied for the dynamic role in normal as well cancer cells. P53 protein is a transcription factor for many cellular proteins such as MDM2, p21, Fas, Bax, p48, PTEN, B99, PAI, etc., involved in cellular transduction. It, directly and indirectly, regulates a wide variety of cellular processes such as apoptosis, senescence, cell cycle arrest, differentiation, DNA repair and replication, and cancer dynamics (7). In normal cells, p53 protein remains in an inactive state. However, due to cellular stress, such as hypoxia, nucleotide depletion, nitric oxide, and DNA damage (8, 9), p53 gets activated, leading to the activation of the various cell- signaling molecules. Various studies reported genetic level interaction between p53 and TLRs (10-12). However,

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molecular interaction studies are still few reported (13).

In the present work, we computationally characterized molecular interaction between p53 and toll-like receptors. We used open web resources for docking and analyzing the data. Our molecular docking and molecular dynamics simulation results suggest that there is a significant interaction between p53 and toll-like receptors. The study could important for the possibility of therapeutic intervention.

Materials and methods

We retrieve conserved sequences for p53 and TLR1 to TLR10 protein in FASTA format from the NCBI database (https://www.ncbi.nlm.nih.gov/). After obtaining these conserved sequences, we did multiple sequence analyses using CLUSTAL Omega, a web tool (https://www.ebi.ac.uk/Tools/msa/clustalo/). After alignment of the conserved region, we used this alignment for phylogenetic analysis. For phylogenetic tree plotting, we used the online available iTOL tool (https://itol.embl.de/)(14). The interaction was downloaded for human p53 and TLR1 to TLR 10 is retrieved from the STRING database (https://string-db.org/). Next, we used the Cytoscape tool, an open web resource(https://cytoscape.org/)(15), to find the interaction between p53 and TLRs protein. We retrieve p53 protein structure data (PDB ID: 2OCJ) from RCSB (https://www.rcsb.org/).Next, we removed unnecessary molecules from the PDB ID:2OCJ structure file by using the discovery studio suit. Further, we also retrieve PDB structure for all human TLRs (from TLR 1 to TLR 10) [PDB ID: 1FYV (TLR1), 1FYW (TLR2), 2MK9 (TLR3), 2Z63 (TLR4), 3JOA (TLR5), 4OM7 (TLR6), 6LVY (TLR7), 5W3M (TLR8), 5Y3M (TLR9), 2J67 (TLR10)].We did protein-protein molecular docking was done using CLUSPRO 2.0 (an open-source tools for protein-protein docking) (https://cluspro.org/help.php) for academic users(16). We selected the best pose provided by docking software. The docking results obtained from CLUSPRO were further analyzed using CABS-flex (an online tool) (http://biocomp.chem.uw.edu.pl/CABSflex2)(17) for molecular dynamics study for 10ns to analyze the stability and flexibility of free p53 protein and TLRs bounded p53 protein.

Results and discussion

The multiple sequence alignment of the conserved region of the protein, which is mostly associated with the interaction between proteins, is shown in Figure 1. We observed a significant consensus base pair.

By the analysis of the phylogenetic tree for the conserved domain, we found out gene ontology and relationship among p53 and TLRs (1 to 10) proteins as shown in Figure 2. Interaction networks among p53 and TLRs (1 to 10) were generated (Figure 3). It is observed that the p53 protein interacts with all the TLRs. A detectable significant interaction network among p53 and TLRs (1 to 10) were found. The interaction score has been listed in Table 1. The molecular docking was done by using the CLUSPRO online server, a very widely known tool for protein-protein docking (16). Moreover, it is also noticed that from the results shown in Table 2 that there is a comparatively strong interaction between p53-TLR3, p53-TLR5, p53-TLR8 and p53-TLR9 complexes. We have shown p53 and TLRs interaction obtained from CLUSPRO online server docking as shown in Figure 4. The root mean square fluctuation (RMSF) defines the mobility of atoms present in a protein structure. The large RMSF values signify a high degree of flexibility and instability of a structure, and a small RMSF value indicates a stable and rigid structure. RMSF of p53 protein was calculated for p53 interacting with each of the TLRs (1 to 10) and without TLRs from the 10ns molecular dynamics trajectories (Figure 5). It is observed that the average RMSF for P53 protein has higher fluctuation due to the binding of TLRs.

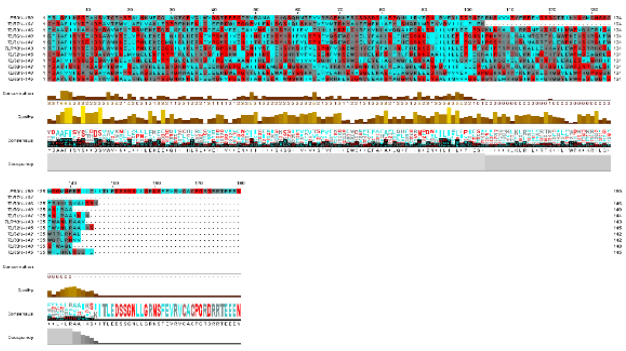

Figure 1. Multiple sequence analysis for the conserved domain of p53 and TLRs protein

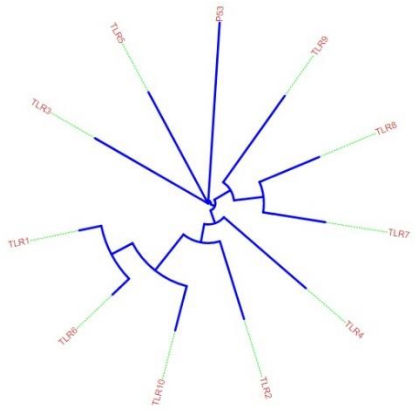

Figure 2. Interactive phylogenetic tree plot for the conserved domains of p53 and TLRs protein

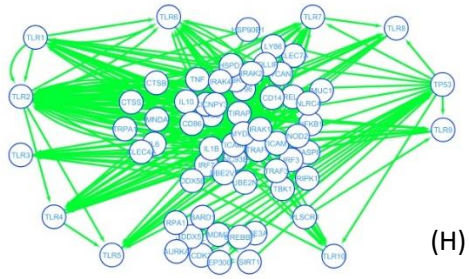

Figure 3. Interaction network between p53 and TLRs protein

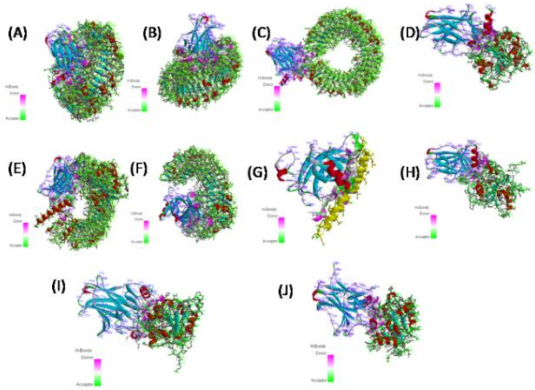

Figure 4. CLUSPRO docking output between p53 and TLRs. (A). Docking between p53 (in blue colour) and TLR 1 (in greencolour) (B). Docking between p53 (in blue colour) and TLR 2 (in greencolour) (C). Docking between p53 (in blue colour) and TLR 3 (in greencolour) (D). Docking between p53 (in blue colour) and TLR 4 ((in greencolour) (E). Docking between p53 (in blue colour) and TLR 5 (in greencolour) (F). Docking between p53 (in blue colour) and TLR 6 (in greencolour) (G). Docking between p53 (in blue colour) and TLR 7 (in greencolour) (H). Docking between p53 (in blue colour) and TLR 8 (in greencolour) (I). Docking between p53 (in blue colour) and TLR 9 (in greencolour) (J). Docking between p53 (in blue colour) and TLR 10 (in green colour)

Table 1. Interaction network score; Name (A), Betweenness Centrality (B), Closeness Centrality (C), Clustering Coefficient (D), Neighborhood Connectivity (E), Radiality (F), Topological Coefficient (G)

A	B	C	D	E	F	G
TP53	0.265004	0.54330709	0.18947368	10.75	0.859903	0.1671875
TLR1	0.018083	0.51111111	0.67619048	19.333333	0.84058	0.32768362
TLR2	0.170667	0.62162162	0.28571429	12.885714	0.898551	0.20453515
TLR3	0.027806	0.46621622	0.74545455	17.727273	0.809179	0.33916084
TLR4	0.037211	0.52272727	0.48366013	17.111111	0.847826	0.29001883
TLR5	0.013799	0.48251748	0.6	17.909091	0.821256	0.30877743
TLR6	0.018083	0.51111111	0.67619048	19.333333	0.84058	0.32768362
TLR7	0.134491	0.51879699	0.26470588	11.941176	0.845411	0.20486815
TLR8	0.028847	0.4893617	0.46666667	13.5	0.826087	0.23275862
TLR9	0.038949	0.5	0.53333333	19.4	0.833333	0.32166667
TLR10	0.011855	0.49640288	0.75	20.555556	0.830918	0.33697632

Table 2. CLUSPRO score for the lowest interaction energy

Complex	CLUSPRO Score (lowest energy in Kcal per mol)
P53-TLR1	-729.3
P53-TLR2	-991.8
P53-TLR3	-1072.7
P53-TLR4	-854.4
P53-TLR5	-1047.6
P53-TLR6	-760.8
P53-TLR7	-996.1
P53-TLR8	-1089.3
P53-TLR9	-1323.3
P53-TLR10	-824.6

The role of p53 in apoptosis and cancer is well reported (7,8,18,19) However, the role of p53 in cellular immunity is still found to be a challenging issue of research (9, 11, 20). In addition, the role of TLRs in cellular immunity is also a very much studied area of research (21-26). Recently, A few reports suggest the role of TLRs in apoptosis and cancer (11, 27-30). Henceforth, there is a possibility for interaction between p53 and TLRs. The consensus

sequence similarity between p53 and TLRs suggests a phylogenetic relationship and possibility for interactions. The interaction network analysis between p53 and TLRs (1 to 10) (Figure 3) suggests that p53 interacts with all the TLRs. Our CLUSPRO docking results (Figure 4) suggest that there are prominent possibilities of interaction between p53 and TLRs (1 to 10) proteins. In addition, it is also noticed that from the results listed in Table 2 that there is a comparatively strong interaction between p53-TLR3, p53-TLR5, p53-TLR8 and p53-TLR9 complexes. The molecular dynamics study for the stability of complexes between p53 and various TLRs proteins also suggests and supports the docking results as shown in figure4. The study can be very helpful to understand the role of p53 in cellular immunity as well as the role of TLRs in apoptosis and cancer regulation. In addition, the study will be used in therapeutic interventions study.

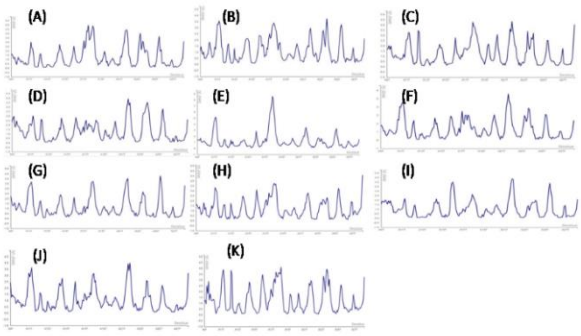

Figure 5. Root mean square fluctuation plot of (A) p53 (B)p53 after bind with TLR1 protein (B) p53 after bind with TLR2 protein(C) p53 after bind with TLR3 protein(D) p53 after bind with TLR4 protein(E) p53 after bind with TLR5 protein(F) p53 after bind with TLR6 protein(G) p53 after bind with TLR7 protein(H) p53 after bind with TLR8 protein(I) p53 after bind with TLR9 protein (J) p53 after bind with TLR10 protein

Conclusions

Here, we computationally characterized molecular interaction between p53 and toll-like receptors proteins. The interactions between p53 and TLRs seem to be significant. The present study suggests a wider role of p53 apart from cell cycle regulation and cancer dynamics. This study can be very useful for molecular therapeutics and drug design strategies against cellular immunity and cancer.

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Interest conflict

The authors declare no conflict of interest.

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