(Online) ISSN 2744-1636
Bioengineering Studies *Original Research*
Vol. 5, No. 1, July 2024, pp.43-51
https://doi.org/10.37868/bes.v5i1.id319

An *in silico* prediction of IRGQ and TRIM21 interactions – autophagy related proteins

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Received Jun. 30, 2024
Revised Jun. 15, 2024
Accepted Jun. 30, 2024

Abstract

Autophagy is an intracellular process that is important for the detection and cleansing of the degraded cellular material, which accumulation can lead to cancer. Autophagy can also be beneficial in the detection and destruction of cancer cells in early stages of cancer, but in late stages, by the implementation of IRGQ protein, it can use the immune system as a tool for enhancing the proliferation and expansion of cancer cells, making the human organism more vulnerable. Implementation of the TRIM21 protein in interacting with IRGQ can be a promising tool to prevent the hostile function of the IRGQ protein and prevent cancer from immune evasion. This paper predicts the 3D structures of these proteins and, by the implementation of docking, reveals the possible immune system enhancement when fighting cancer.

Keywords: TRIM21, IRGQ, in silico, protein-protein interaction, immune system

1. Introduction

The Immunity-Related GTPase Q (IRGQ) protein is a member of a group of proteins that are crucial when it comes to innate immune responses. IRGs, as a protein family, are characteristic because they are highly conserved across mammals. They are originally recognized as the main characters when it comes to host defense against pathogens. The mechanism that they use regularly for defense is modulating vesicular trafficking, autophagy, and phagosome maturation. This protein is expressed across various tissues, including the immune and non-immune cells, which offer a broad functional scope that can be beyond traditional immune defense mechanisms [1].

IRGQ protein can be defined as a newly discovered protein, and its function and definition are still a matter of research. But, until today, the importance of this protein has not been defined, and what is crucial for the expansion of research, the area for prediction and connection to other proteins has been expanded.

Most recent studies have presented that the IRGQ protein can be a pivotal factor when it comes to the modulation of interferon-mediated immune responses, which can eventually influence the activation of immune cells [2].Because of the novelty of this protein, the field of research remains free and under the influence of the connection to the proteins with similar function, and also, what is most important, providing the connection to the non-related proteins but with the functions that can benefit and may be a turning point to curing diseases when interacting with the IRGQ protein [2].

1.1. Autophagy-self-healing mechanism

The modern world puts humans under an enormous amount of stress and pressure, which can cause serious damage on a cellular level. The majority of organelles get damaged, but one of the highest risk is mitochondria, and ultimately, it's the specific DNA called mitochondrial DNA. Accumulation of damaged mitochondrial DNA (mtDNA) can lead to a serious disease, cancer. Cancer is an end-stage disease that happens when the body is not cleansing enough, and the accumulation of damaged cell materials and damaged cells in general leads to the formation of "zombie cells, " also called tumor cells [3]. Cleansing of the damaged cells can be simply done with the process of autophagy. Autophagy is a lysosome-mediated process whose main goal is the degradation and recycling of damaged cellular material. It serves as a crucial tool for orchestrating the responses to metabolic stress, microbial infections, and fighting autoimmune diseases and cancer [2][3].

1.2. Dual role of autophagy in cancer

Autophagy can be a very beneficial tool for improving health and preventing diseases, particularly when an individual is under constant stress. This makes it a perfect candidate for the accumulation of damaged cellular material, which can then progress into cancer cells. Applying fast as a motor that switches on autophagy can firstly be useful for the prevention of diseases such as cancer, but also for cleansing the organism from cellular debris [4]. Autophagy is a process very valuable in early stages of cancer since by implementing healthy code, it can signalize the protein complexes to initiate the autophagosomes production, which can then localize the damaged cells or cancer cells and use them as a fuel for the body, lowering the number of tumor cells and preventing metastasis [4][3]. On the other hand, as cancer progresses to stages 3 and 4 and metastasis starts to rise, the implementation of autophagy in that case can be very delicate. The opposite effect can be achieved, posing a threat to healthy cells and promoting the development of cancerous ones. Autophagy in late stages of cancer can initiate the proliferation of cancer cells and make the patient more vulnerable to chemotherapy, increasing the number of side effects that can shorten lifespan and make the disease worse. All in all, careful implementation of autophagy in early stages of cancer and in healthy individuals can be crucial for the prevention of cancer, because of its role in cleaning of damaged cellular material, particularly mtDNA, the main cause of cancer development in numerous [5].

2. Materials and methods

2.1. Retrieving sequences of IRGQ and TRIM21

To obtain sequences in FASTA format, it was first important to use the UniProt online database to obtain the UniProt IDs of these proteins, as shown in Table 1.

Table 1. Retrieving protein sequences from UniProt

Protein	UniProt ID
IRGQ	8Q6Q
TRIM21	P19474

2.2. Prediction of 3D structure models and their validation

The SWISS model server is a structural bioinformatics software for homology modeling of protein 3D structures. It is used to generate PDB (Program data base) files online [6]. Furthermore, the 3D structure of each subunit was verified via Ramachandran plots using the RAMPAGE program [7].

The additional validation and stereochemical analysis of the predicted structures of the docking model is done by the integrated verification tools from the SWISS MODEL server. One model with the best QMEAN and highest coverage was selected for this study. Three-dimensional models and Ramachandran plots are presented in the results section.

2.3. Docking site prediction

Docking is a method used to model protein-protein complexes in quaternary structure by implementing two or more biological macromolecules. ClusPro 2.0 is a server used to predict docking sites of IRGQ and TRIM21. In order to complete docking, PDB files of selected 3D models from SWISSMODEL are gathered and placed into ClusPro 2.0, and a graphical representation as well as a table with scores are presented in the results section.

3. Results

3.1. Predicted 3D models by SWISSMODEL

In order to obtain 3D models, first, it was necessary to get the FASTA format of proteins and put it in software to predict models. Based on the highest coverage and QMEAN, one model was picked and presented for each protein.

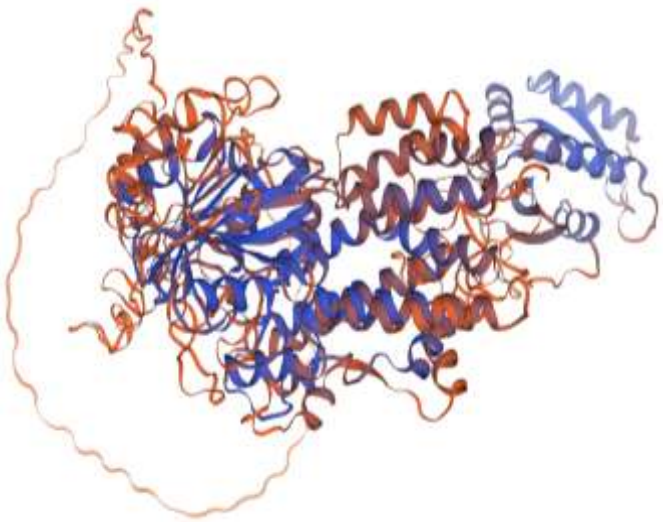

Figure 1. Predicted 3D model for IRGQ protein

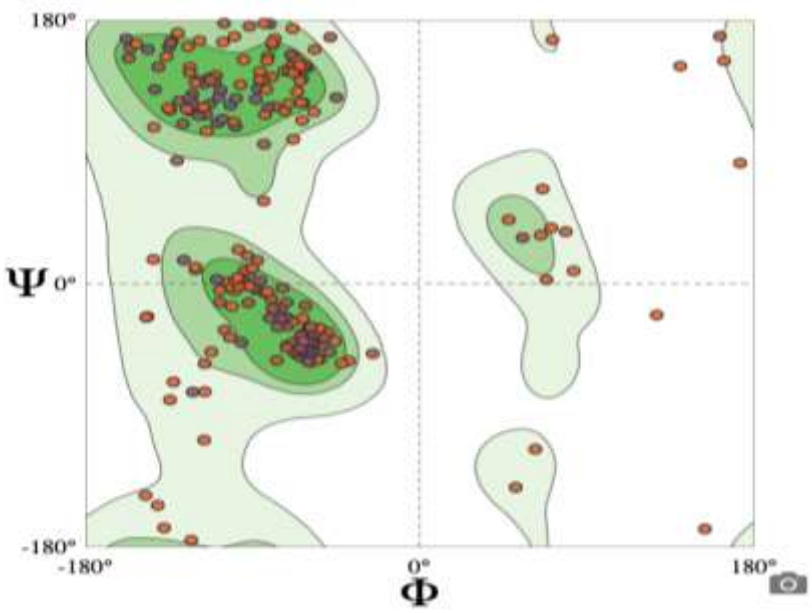

Figure 2. Ramachandran plots for the predicted model of the IRGQ protein

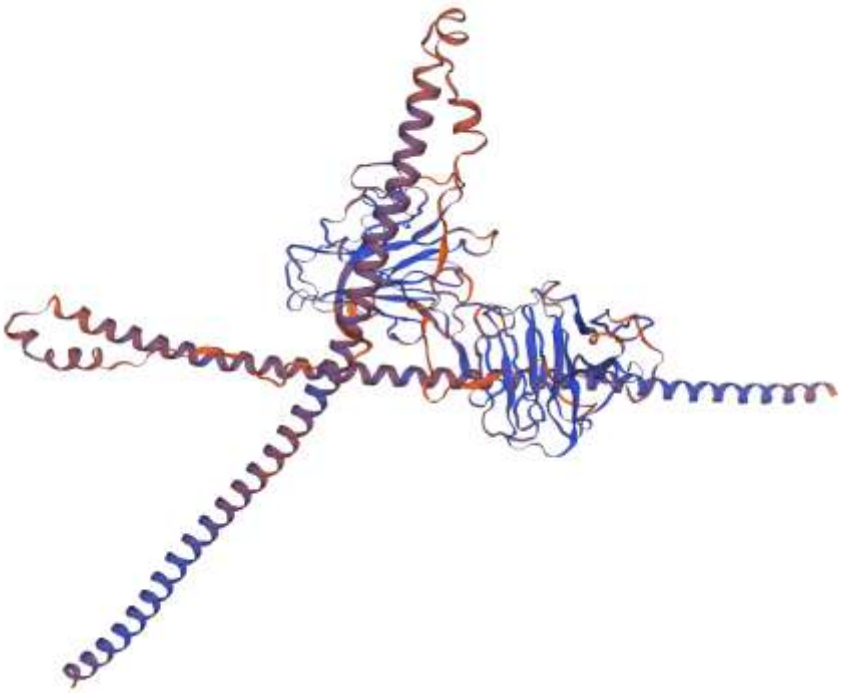

Figure 3. Predicted 3D model for TRIM21 protein

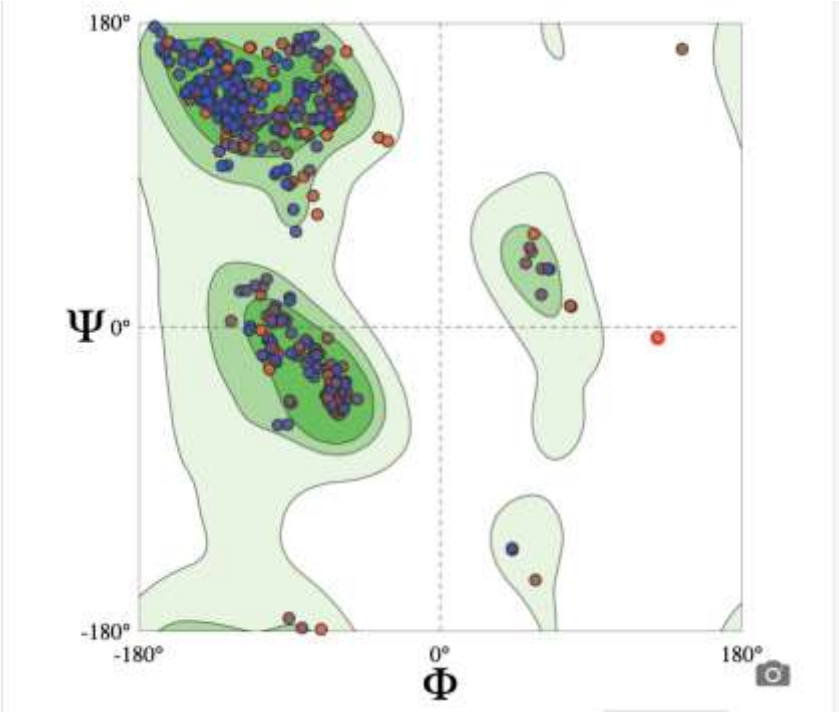

Figure 4. Ramachandran plots for the predicted 3D model for the TRIM21 protein

3.2. Molecular docking using ClusPro 2.0

Results of molecular docking of proteins IRGQ and TRIM21 **(**Figure 5.), as well as the table (Table 2.) with designated scores, were presented in the following figure.

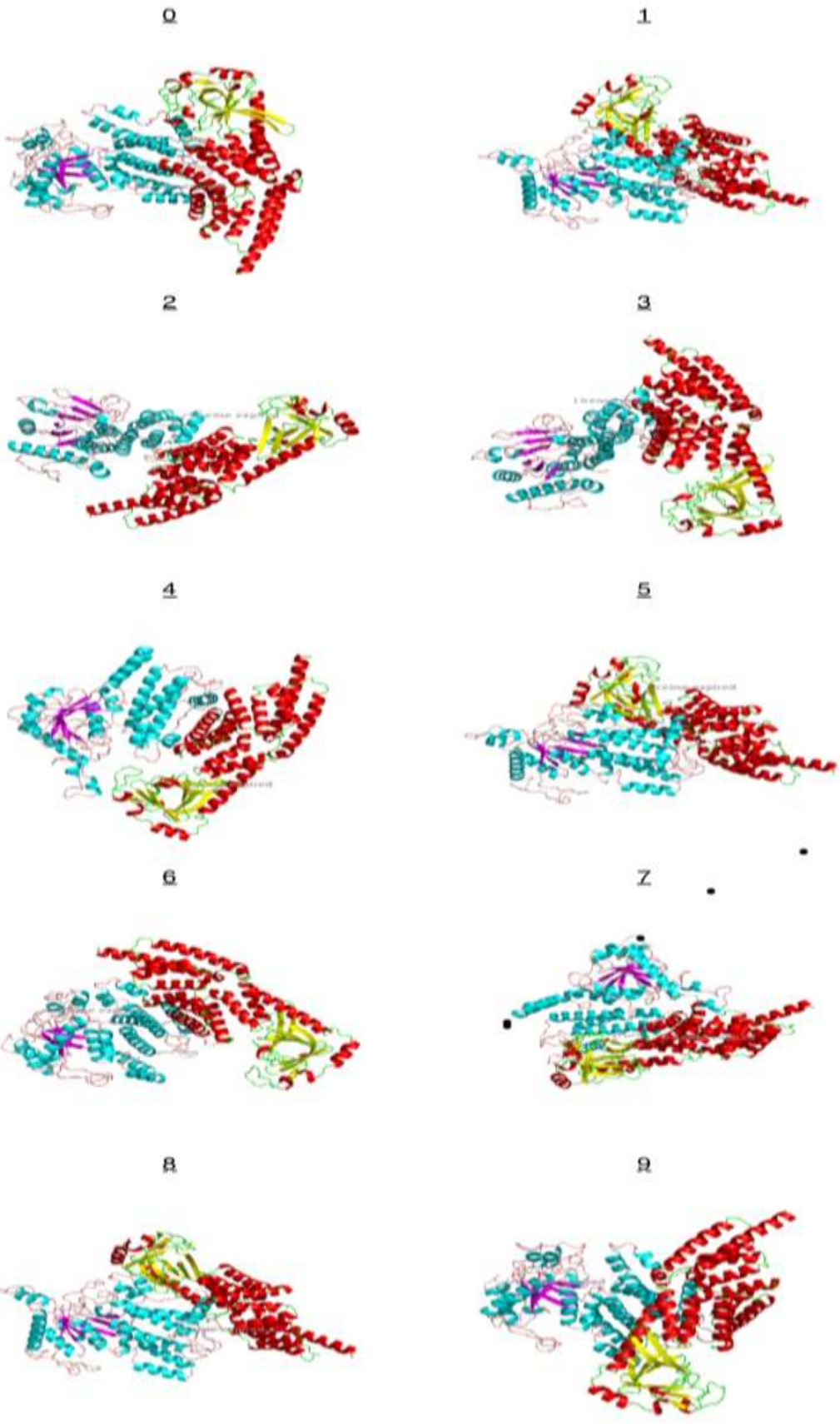

Figure 5. Docking of IRGQ and TRIM21 protein using ClusPro 2.0

Table 2. ClusPro 2.0. docking results with weighted scores

Cluster	Members	Representative	Weighted Score
0	105	Center	-1025.9
0	105	Lowest Energy	-1025.9
1	85	Center	-912.5
1	85	Lowest Energy	-1093.2
2	74	Center	-810.6
2	74	Lowest Energy	-985.6
3	56	Center	-812.8
3	56	Lowest Energy	-955.1
4	56	Center	-961.4
4	56	Lowest Energy	-1011.1
5	42	Center	-807.8
5	42	Lowest Energy	-1055.6
6	35	Center	-863.9
6	35	Lowest Energy	-915.8
7	33	Center	-972.0
7	33	Lowest Energy	-1021.5
8	26	Center	-820.6
8	26	Lowest Energy	-927.6
9	23	Center	-801.3
9	23	Lowest Energy	-1005.3
10	19	Center	-959.3
10	19	Lowest Energy	-959.3
11	18	Center	-777.8
11	18	Lowest Energy	-874.4
12	18	Center	-776.3
12	18	Lowest Energy	-862.5
13	17	Center	-864.4
13	17	Lowest Energy	-926.1
14	16	Center	-785.6
14	16	Lowest Energy	-1091.5
15	16	Center	-828.1
15	16	Lowest Energy	-831.4
16	16	Center	-835.2
16	16	Lowest Energy	-854.3
17	14	Center	-791.6

17	14	Lowest Energy	-879.8
18	14	Center	-877.6
18	14	Lowest Energy	-877.6
19	13	Center	-825.7
19	13	Lowest Energy	-972.6
20	13	Center	-824.3
20	13	Lowest Energy	-896.7
21	13	Center	-806.0
21	13	Lowest Energy	-899.6
22	12	Center	-819.5
22	12	Lowest Energy	-854.0
23	12	Center	-854.8
23	12	Lowest Energy	-889.1
24	12	Center	-845.0
24	12	Lowest Energy	-868.6
25	11	Center	-818.3
25	11	Lowest Energy	-953.2
26	10	Center	-932.9
26	10	Lowest Energy	-932.9
27	10	Center	-831.6
27	10	Lowest Energy	-831.6
28	10	Center	-803.6
28	10	Lowest Energy	-867.0
29	10	Center	-782.7
29	10	Lowest Energy	-815.5

4. Discussion

4.1. Including the TRIM21 in the autophagy complex

TRIM21 plays a significant role in autophagy, regulating it using ubiquitination of key proteins. TRIM21 is an autophagy-interacting protein that interacts with autophagy receptors by modulating immune responses, detecting the misfolded proteins or pathogens, making them candidates for lysosomal degradation. This protein is influencing the autophagosome formation through mechanisms such as K63-linked ubiquitination [8].

4.2. Correlation of TRIM21 and IRGQ as a novel approach in regulating autophagy

TRIM21 is a protein that recognizes misfolded or cytosolic proteins, while IRGQ is a GTPase with exposed domains amenable to ubiquitination. TRIM 21 has the potential to ubiquitinate IRGQ, marking it for proteasomal degradation. This interaction would reduce the IRGQ protein levels, preventing the interaction with GABARAPL/LC3B and inhibiting the MHCI class degradation, making it a perfect mechanism for the immune system switch from hostile to normal, particularly in cases of autophagy in late stages of cancer [8].

4.3. Predicting the possible interaction of IRGQ and TRIM21 in the context of cancer treatment

When it comes to the cancer relevance of these two proteins, their functions can be very interesting since, as a potential protein complex, they can be very beneficial in cancer treatment. Because they are both involved in autophagy, they could work as potential partners. IRGQ is predicted to bind GTP, and it is involved in autophagy by mediation of quality control of major histocompatibility complex class I (MHC class I) molecules by promoting detection and degradation of misfolded or damaged MHC class I molecules to facilitate tumor immune evasion, which is found especially in hepatocellular carcinoma [9].

4.4. Docking results revealing

Scores presented in Table 2. are reflecting the energy of the cluster centers, using the calculation from the PIPER docking program. The lowest, most favorable score recognized from the table was -1093, and the highest was -785, the least favorable. These scores represent the interaction energies between two proteins, calculated by the PIPER docking algorithm, which also evaluates the van der Waals, electrostatic, and desolvation contributions. Also, what is important to mention is that the lower the score, the more favorable synergy of the two proteins is, so cluster with weighted score -1093 reflects a highly favorable interaction between IRGQ and TRIM21. Additionally, it is necessary to go further with the more important cluster sizes, so cluster 0 and cluster 1 have the most members (105, 85), which can also indicate the high energy as the cluster expands.

5. Conclusion

The ClusPro results provide a robust starting point when it comes to understanding the IRGQ protein and TRIM21 protein, as well as their possible interaction in terms of autophagy. The clusters with the lowest scores are favorable for future research and further reveal the synergy of these two proteins. This alone can be beneficial in introducing novel concepts of treatment, as well as understanding the novel protein, particularly since the discovery of IRGQ protein dates to 2024. Results provided from this research paper were obtained from a limited amount of literature, due to the knowledge of this protein being finite. Moreover, introducing the potential connection to the TRIM21 can be a unique concept in the field of autophagy and the correlation between autophagy and the immune system, since these two proteins share their involvement in immune response as well as autophagy. This research paper might be the first of its kind since there was no correlation between TRIM21 and IRGQ recorded before, which can be presented as a promising tool for understanding cancer, immune evasion, and autophagy as the holy trinity nowadays.

Declaration of Competing Interest

The authors declare that they have no known financial or non-financial competing interests in any material discussed in this paper.

Funding information

No funding was received from any financial organization to conduct this research

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