European journal of volunteering and community-based projects Vol.1, No 2; 2021
ISSN: 2724-0592 E-ISSN: 2724-1947
Published by Odv Casa Arcobaleno

The Immunoinformatic, Structural elucidation of ULBP2 Protein in the therapeutics of Tumorigenesis: Using Bioinformatics Approaches

Muhammad Mazhar Fareed

Faculty of Life Sciences, Department of Bioinformatics and Biotechnology, Government College University, Faisalabad, Pakistan

Khazeema Yousaf

Department of Biotechnology, Lahore College for Women University, Lahore, Pakistan

Muhammad Salman Akber

Faculty of Life Sciences, Department of Bioinformatics and Biotechnology, Government College University, Faisalabad, Pakistan

Muhammad Mohsin Ali

Faculty of Medical Sciences, Department of Physical therapy, Government College University Faisalabad, Pakistan

Abstract: The Natural killer (NK) cells' ability to destroy cancerous cells is predominantly focused on the activation of the co-stimulatory and natural killer group two with receptor of member-D also called NKGD2/NKG2D. These identifies ligands that are MHC-Class1 structural homologs like that of the UL16 protein-binding type 2. The ULBP2 has been shown to mediate-natural resistance against the tumors mechanism in the condition of in-vitro, in-vivo, making it a possible target for producing the immune-therapeutic drugs for the diagnosis of the cancers and certain other viral diseases. In this present research, we created a stable and high-quality 3-D structure of the ULBP-2 protein through SWISS-Model also visualized by using UCSF-Chimera Tool. Moreover, the ULBP2 protein was prognosticated to be acts as antigenic, 11-discontinuous-B-Cell epitopes, 05 ULBP2 proteins antibody-based epitopes, and possible predicted the top hits of six linear B-cell epitopes. The ULBP2 protein carried seven cytotoxic-T lymphocytes (CTLs), two helper-T lymphocytes (HTLs), the LGKKLNVTTAWAQN is a promiscuous epitope MHC bounded to the T cells and with LRDIQLENY highest antigen scores in MHC molecule. Finally, the promising epitopes that could be successful in producing B-cell and T-cell mediated immunity against the required immune reaction to tumorigenesis were expected.
Keywords: Cancer, B cell epitope, ULBP2, T cell epitope, bioinformatics

Doi: 10.5281/zenodo.5035599

1.Introduction

Cancer becomes a worldwide threatened disease amongst to the other non-communicable diseases and it harm the normal cells instead of abnormal proliferating cancerous cells. Moreover, it becomes the largest diseased population since in lower and mediocre population countries (DeSantis et al., 2014; Ma & Yu, 2006). For tumor immunotherapy which is based on (Natural Killer 2D) NKG2D the cancer owns vital importance. It is studied that to fight immune systems to transformed cells the natural killers are the best cells which play key role to helping the immune system as well as innate of immunosurveillance of cancer (Shulman & Mok, 2015).

In immune system ULBP2 are the key contributors depends upon signalling molecules identify of harmful agents that causes problems in body. In carcinomas, there are three ligands i.e., ULBP2, MICB and MICA of NKG2D which are mostly manifest. It has been under consideration from past few decades that the vaccine against tumor immunology but it is inappropriate for several reasons e.g., the targeted antigenic cells are more competent to the vaccine so vaccine does not react properly (Marcus et al., 2014; Mistry & O'Callaghan, 2007).

For immunotherapy NK2D ligands are targeted which are consider as primary tumors ligand and this ligands NKG2D are the protein complexes and composed of natural killer cells, subsets of T cells, NKT cells, activated γ δ T cells and CD8+αβ T cells. NKG2D is found to be elevating the immunity to remove ligands by activation of lymphocytes and it is present in human cells having 8 ligands on the surface of the healthy cells and only manifest when mutation, deterioration and in stressed situation occurs. This type of situation makes pathways to control manifestation of the cells at various states (Fleri et al., 2017; Raulet, Gasser, Gowen, Deng, & Jung, 2013).

In humans the ULBPs (Unique long 16 binding proteins) or RAET (retinoic acid transcript) with an encoded gene RAET1 are the ligands for NKG2D receptors. These are only express in tumor cells and play vital role in biological functions of NK cells. ULBP2 (Unique long 16 binding-proteins) are found to be having potential to normalize the resistant tumor infections agents *in vivo* which we can uses as immunotherapeutic agents for the treatment of cancer and such fetal viral infection disease. So, we can say that ULBP2 are the most prominent ligands of NKG2D (Fleri et al., 2017; Li et al., 2009).

Due to malfunctioning or failure of NKG2D ligands including flaking and downregulation from the cell surface and loss in the triggering immunes response protein (HLA) or up regulation of non-classical human leucocytes antigen-1 and other proteins which are responsible for activation of immune system and these two factors are attributed in literature (Fleri et al., 2017; McGilvray et al., 2010). Prior to it now the major priority is to making vaccine having insight into the ULBP2 and epitope sites (Protein in β cells and T cells) by computational methods. Vaccine designing is becoming the major issue and it is done on the computational analytical tools. These studies proposed that we find the potent target of ULBP2 protein against the analysis of cancer for the effect of the epidemiological and genetic characteristics in cancer diseases of the population.

2.Material and Methods:

2.1 Finding sequence

We reviewed the literature and choose the ULBP2-Protein because of its different immunogenic functions in cancer development, as mentioned in the introduction section. In

order to assess ULBP2 as a potential cancer therapeutic option, we obtained the amino acid sequence in FASTA format from the NCBI (National Center for Biotechnology Information protein database) (https://www.ncbi.nlm.nih.gov/).

2.2 Analysis of protein structure

According to the research work of this given protein target sequence, the prediction of physicochemical properties (secondary structure) of ULBP2 was prognosticated using the Protparam_Tool (https://web.expasy.org/protparam/) and the 2D-protein structure through SOPMA Tool (Geourjon & Deleage, 1995), the predicted of coiled coil region of protein through ExPASY-COIL (Gasteiger et al., 2003), transmembrane helices of ULBP2 protein were predicted through TMHMM Server v. 2.0 (http://www.cbs.dtu.dk/services/TMHMM/), the protein family's prediction by using pfam-database (Bateman et al., 2004), and the ULBP2 protein association network analysis was performed through STRING-Database (Szklarczyk et al., 2016).

2.3 3D structure prediction and model refinement, evaluation

The ULBP2 targeted protein structure prediction via using template-model of PDB-ID: 4S0U through SWISS-Model (Schwede, Kopp, Guex, & Peitsch, 2003), and the respective protein target structure were appraised using the SAVES v6.0–UCLA Tool (https://saves.mbi.ucla.edu/), Molprobity-Rampage Tool (Chen et al., 2010), the model refinement through ModRefiner-Zhang_Lab (https://zhanglab.ccmb.med.umich.edu/ModRefiner/), the refined structure need to be more via based on experimental validation were applied in PROSA Web Tool (Wiederstein & Sippl, 2007). And the predicted structure visualized through UCSF-Chimera Tool, and also visualized domain of ULBP2 (Pettersen et al., 2004).

2.4 Immunoinformatic profiling of target protein

In immunoinformatic approaches, antigenic and epitope prediction sites, in which we investigated the ULBP2 protein antigenicity through Vaxijen v2.0 server (Doytchinova & Flower, 2007), and antigens protein prediction by using Universidad Complutense Madrid Immunomedicine Group antigenic prediction tool. This server working principle by protein sequence segments within the protein possible most antigenic region to stimulate the mechanism of antibody action in body with the predicting-antigenic almost 75 percent in ranged (Álvarez, Gomez, Mercado, Ramírez, & Marshall, 2016; EL-Manzalawy, Dobbs, & Honavar, 2008). The prediction of the given sequence of ULBP2-based B_cell epitopes was analyzed through BCPRED Tool with the using parameters as by default: set to twenty and percentage of specificity 75%. The prediction of discontinuous B-cell epitopes residues from the three dimensional protein was observed through DISCOTope_Server (Haste Andersen, Nielsen, & Lund, 2006).

The epitope-based checking of the HTL and CTL of the given targeted protein sequence predicted structure of ULBP2 was performed through NETCTL-server to get the MHC_Class1 in the domain of epitope of CTL and, the MHC_Class2 epitopes of HTL were analyzed through NETMHC2pan-server (Scholz et al., 2017) with the values parameter's based: 2%, protein peptide binding: 10 percent. Finally, the obtained protein sequences of HLA_T-Cell's epitope characteristics as; allergens or non-allergen through ToolAllerTOP v. 2.0 (Dimitrov, Bangov, Flower, & Doytchinova, 2014). The respective protein membrane orientation of tertiary structure was analyzed by using OPM-Server (Lomize, Pogozheva, Joo, Mosberg, & Lomize, 2012), the linear epitope antigenic prediction of ULBP2 protein was predicted through SVMTrip Tool (Yao, Zhang, Liang, & Zhang, 2012). The ULBP2-protein structure pocket

regions and cavities with the structural and binding specific regions of protein and nucleic acid were identified through server- GHECOM (Grid-based HECOMi finder), and protein antibody epitope prediction of ULBP2 model were analyzed from ElliPro_IEDB-analysis resource (Ponomarenko et al., 2008). As a result, a prospective vaccine model must also be assessed for benefits and risks.

3.Results:

The physiochemical properties of the ULBP2 protein as results; all the 20 amino acids in the target protein with molecular weight (MW) of 23,655.29 Dalton (Da) are shown in table 01.

Table1: Physicochemical properties of ULBP2

ProtParam Parameters	**Values of ULBP2**
Number of amino acids	210
Molecular weight	23655.29 Da
Theoretical pI	6.52
Atomic composition	Carbon C 1060 Hydrogen H 1648 Nitrogen N 282 Oxygen O 304 Sulfur S 14
Formula	$C_{1060}H_{1648}N_{282}O_{304}S_{14}$
Number of negatively charged residues	24
Number of positively charged residues	23
Extinction coefficient	44835 Abs 0.1% (=1 g/l) 1.895, assuming all pairs of Cys residues form cystines 44460 Abs 0.1% (=1 g/l) 1.879, assuming all Cys residues are reduced
Total number of atoms	3308
Estimated Half-life	30 hours (mammalian reticulocytes, *in vitro*). >20 hours (yeast, *in vivo*). >10 hours (Escherichia coli, *in vivo*).
Aliphatic index	79.43
Grand average of hydropathicity (GRAVY)	-0.233
Instability index	47.67

And widely contributed in the structure of amino acid abundant in the sequence; 11%, tyrosine-1.9%, theoretical-Pi Value: 6.52 with acidic in nature are shown in figure 01.

European journal of volunteering and community-based projects Vol.1, No 2; 2021
ISSN: 2724-0592 E-ISSN: 2724-1947
Published by Odv Casa Arcobaleno

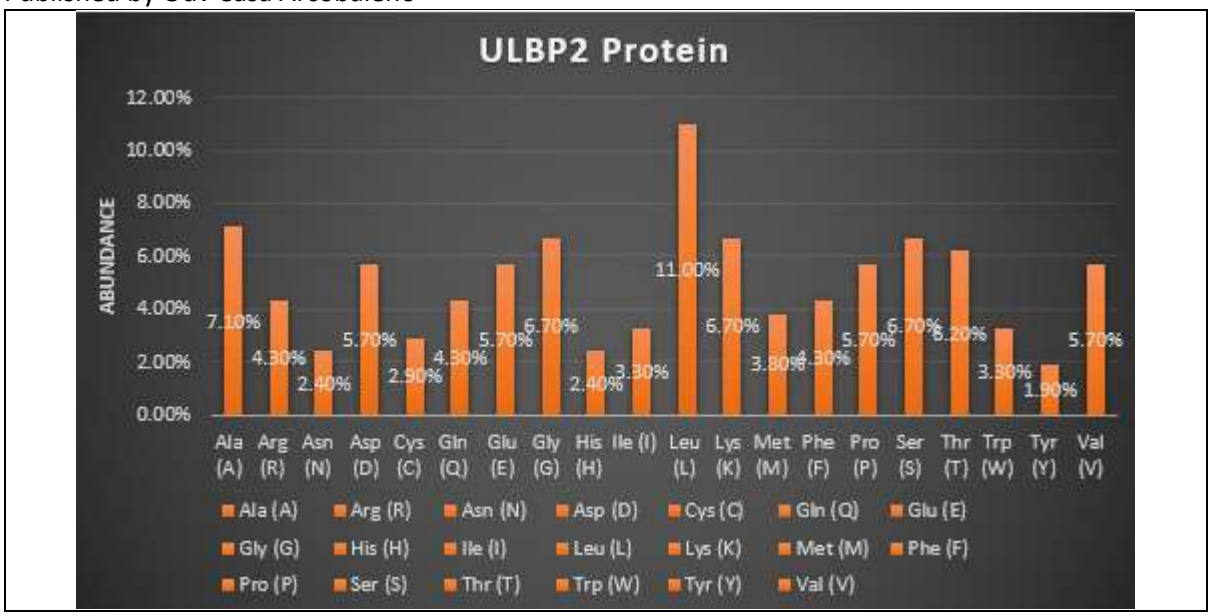


Figure no 1: Amino Acid Composition of ULBP2 with the range between highest to lowest abundance with amino acid percentage (%)

The secondary-structure of the coordinative protein model were through SOPMA_Tool are results: Composition: Alpha helix (Hh): 104 is 42.28% 310, helix (Gg): 0 is 0.00%, Pi helix (Ii): 0 is 0.00%, Beta bridge (Bb): 0 is 0.00%, Extended strand (Ee): 44 is 17.89%, Beta turn (Tt): 23 is 9.35, Bend region (Ss): 0 is 0.00%, Random coil (Cc): 75 is 30.49%, Ambiguous states (?): 0 is 0.00%, Other states:0 is 0.00%, and also shown in figure 02.

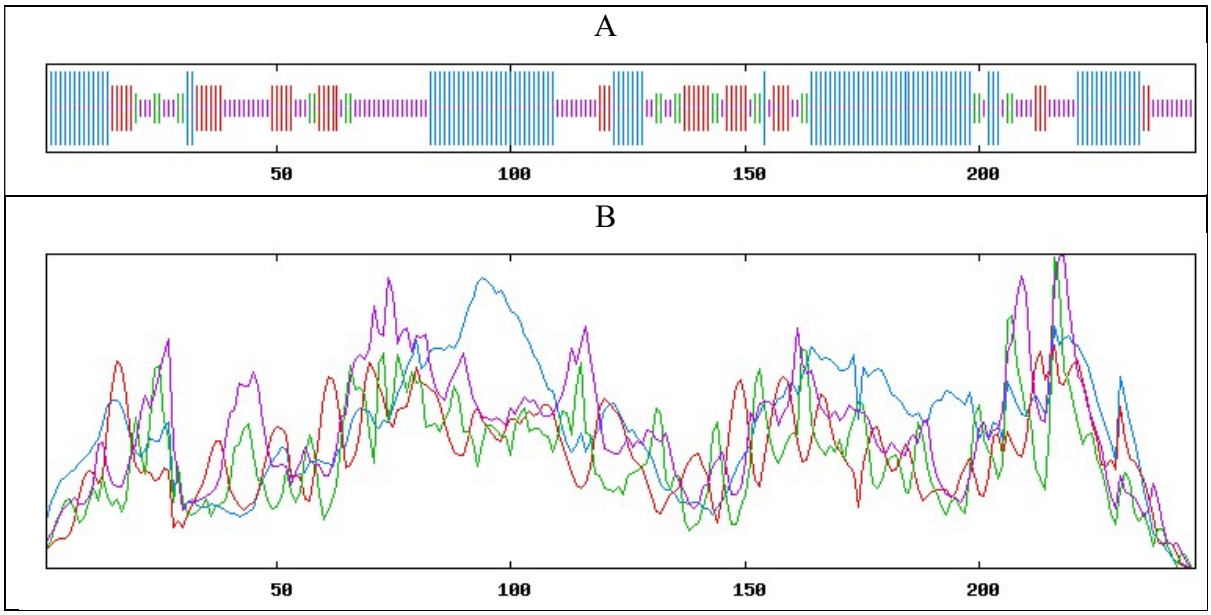


Figure no 02: The secondary structure of targeted protein of ULBP2**: A:** structural, no of amino acid with different color. **B:** highlighted with color like: alpha-helix, pi-helix: dark-blue, beta bridge, extended strand: red, beta turn: black, bend region, random coil: orange, others: green

The protein inside coils prediction through Coil-ExPASY Tool given results are shown in figure 03.

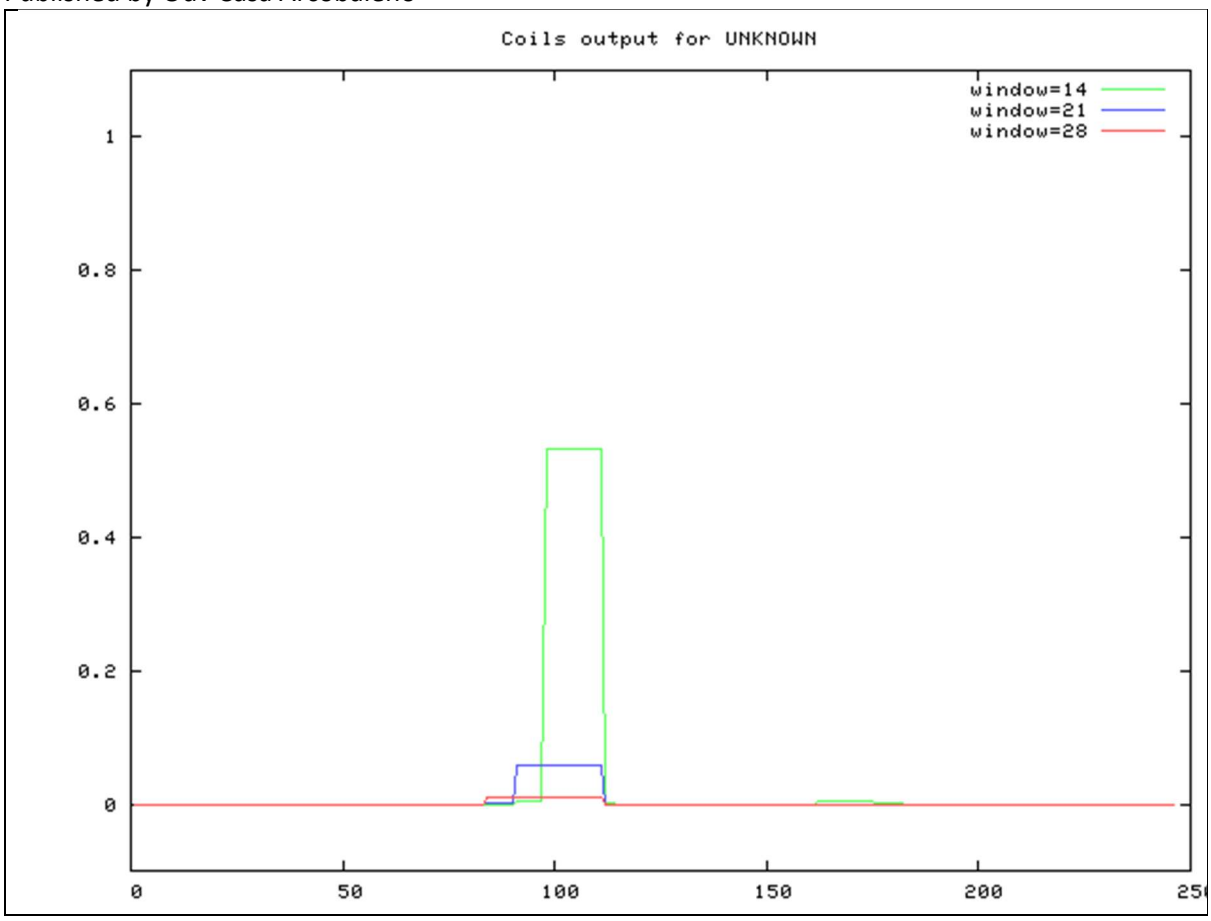
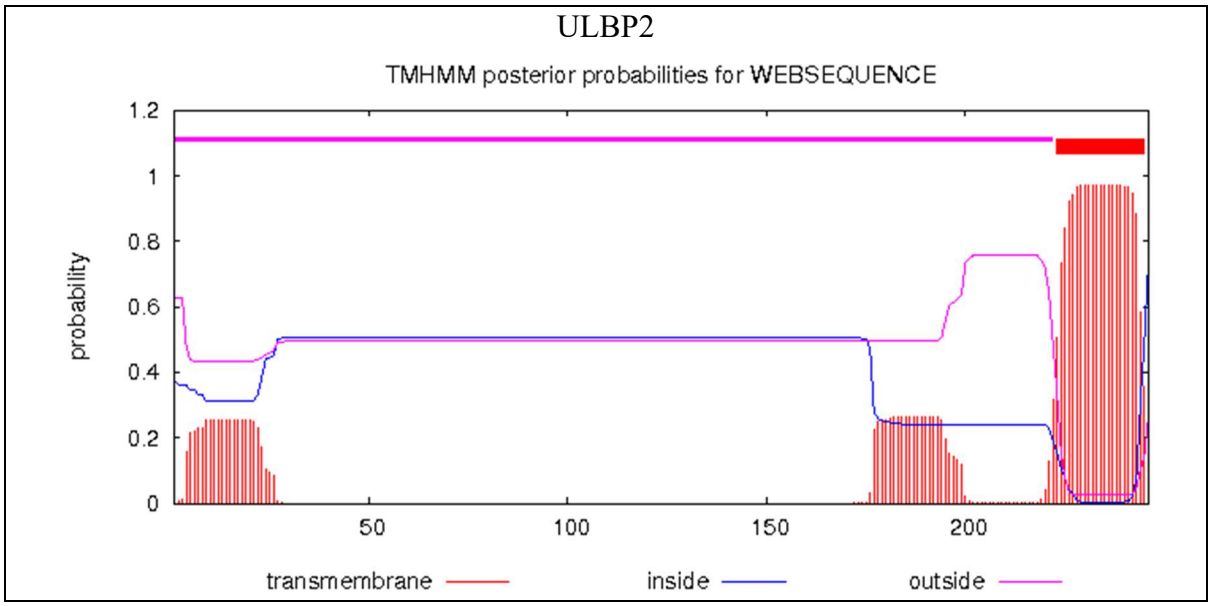


Figure no 03: The Coiled-Coils prediction of ULBP2 protein are showing with the help of graph lines (**Pink, Green, Blue** with color range between 0.00-0.5, 80.00-110.00)

The transmembrane helices of ULBP2 protein with the results; no. of predicted TMHs:1, Exp-no. of AAs in TMHs: 31.34989, Exp-no. of 1st 60 AAs: 5.08604, Total probabilities of N-in as: 0.37142 are highlighted in figure 04.



European journal of volunteering and community-based projects Vol.1, No 2; 2021
ISSN: 2724-0592 E-ISSN: 2724-1947
Published by Odv Casa Arcobaleno

Figure no 04: The ULBP2 Protein of transmembrane-helices with color shows: transmembrane-helices: **RED**, inside: **BLUE**, outside: **PINK**

The ULBP2-protein of domain in the family of MHC-1, Class1- histocompatibility antigen with alpha domian1-2: envelop ranges: 29-205, alignment: 30-199, HMM: 2-173, HMM length: 179, bit score: 69.00, E-value: 3.7e-19 through pfam database. The ULBP2 protein association network was analyzed through STRING Database with some parameters: no. of nodes, edges/expected edge: 11-45/10, average: 8.18, avg clustering-coefficient: 0.919, and PPI p-value: 2.33e-15 along the ULBP2 Protein-protein network are shown in figure 05.

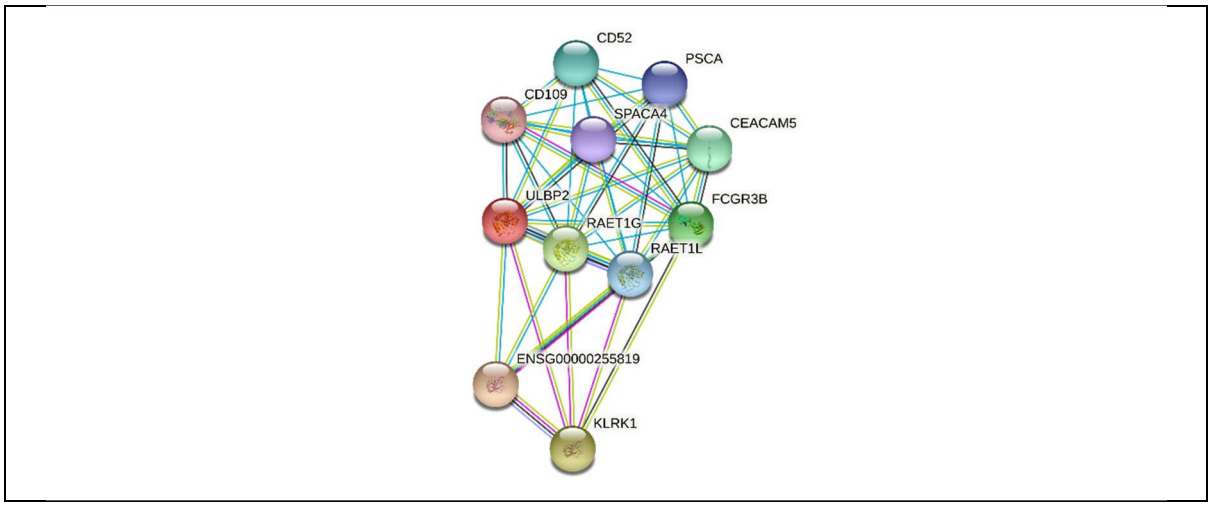


Figure no 05: The STRING Protein network analysis of ULBP2

The validation and qualities observation of obtained protein predicted structure of ULBP2 were observed by using SAVES tool, in ERRAT: results as an overall quality factor are 77.84%, QMEAN-Swiss Model scores of -1.02 with made up of linear combination of amino acid, and the Molprobity scores: 0.86, Ramachandran region: 95.95%, rotamers outliers: 1.27%, and the predicted 3D-structure of ULBP2 through SWISS-Model was visualized with UCSF-Chimera Tool, and structural validation with the score-z are -7.68 are shown in figure 6 (part A, B).

A

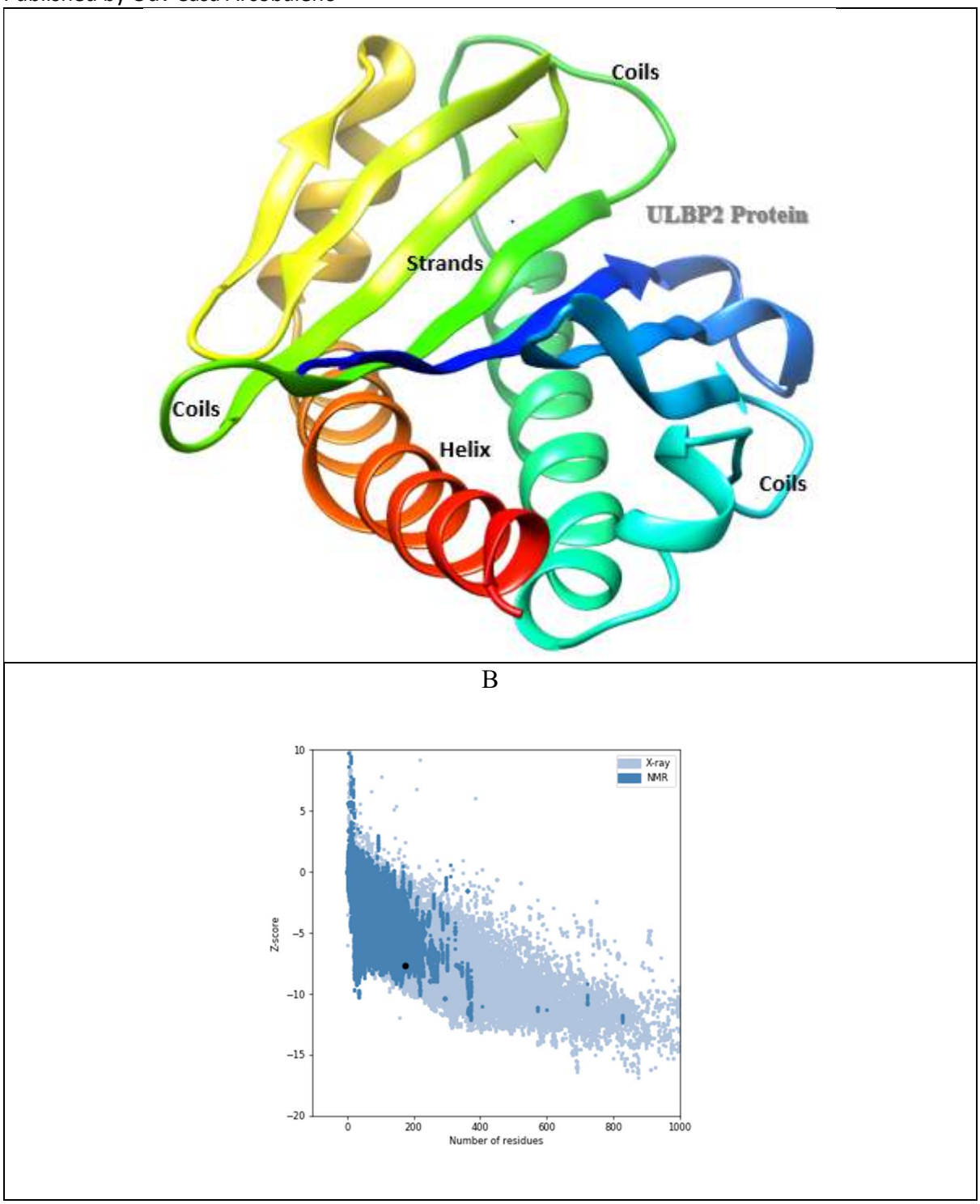


Figure no 6: The ULBP2 protein 3D model showing along the helices, coils and strands with Part A, Validated structure of ULBP2 through Prosa-Web with z-score: -7.68 with Part B

The ULBP2 protein was determined to become a likely tumour antigen by the Vaxijen antigen prediction server, with an antigenicity score of 0.5128, and the ULBP2 sequence contains eight antigenic epitopes as shown in table 02.

Table no 02: The Predicted antigenic peptides on ULBP2 with average antigenic propensity of 1.0294

Start Position	Sequence	Position End
4	AAATKILLCLPLLLLLS	20
29	DPHSLCYDITVIPK	42
46	GPRWCAVQG	54
57	DEKTFLHYD	65
70	TVTPVSPLGKKLNV	83
89	AQNPVLREVVDILTE	103
143	FDGQIFLLF	151
174	NDKVVAMSFHY	184

The BCPred predicted six linear B-cell epitopes within the ULBP2 query protein and classified these based on their ratings are shown in table 03.

Table no 03: The predicted linear B cell epitopes and scores

Position	Antigenicity-epitope	Scores
35	YDITVIPKFRPGPRWCAVQG	0.997
61	FLHYDCGNKTVTPVSPLGKK	0.989
159	TTVHPGARKMKEKWENDKVV	0.971
108	IQLENYTPKEPLTLQARMSC	0.91
82	NVTTAWKAQNPVLREVVDIL	0.89
130	KAEGHSSGSWQFSFDGQIFL	0.816

The DiscoTope server predicted conformational dislocation B-cell epitopes based on the 3-D structure of ULBP2. In total, 20 amino acid residues outside from 210 were reported as discontinuous B-cell epitopes with the specificity, sensitivity values: 0.75-0.47, and threshold values: -3.7 are shown in table 4.

Table no 4: The prediction of B-cell epitopes based on the 3-D structure of ULBP2

Residues No.	Amino acid No.	No. of Contact	Scores of propensities	Score of Disco-Tope
29	Asp	3	-3.204	-3.181
46	Gly	6	-3.321	-3.629
109	Gln	4	-2.467	-2.643
111	Glu	14	0.148	-1.479
112	Asn	2	1.206	0.837
113	Tyr	24	-0.193	-2.931
114	Thr	2	-1.075	-1.181
116	Lys	7	-3.086	-3.537
117	Glu	1	-2.902	-2.684
131	Ala	0	-1.185	-1.049
132	Gly	3	-0.751	-1.010
133	Gly	1	-3.067	-2.830
134	His	5	-2.735	-2.996
163	Pro	1	-0.529	-0.583
164	Gly	10	-0.840	-1.894
166	Arg	4	-1.033	-1.374
167	Lys	12	-0.494	-1.817

170	Glu	8	-0.959	-1.769
171	Lys	18	-1.010	-2.964
174	Asn	6	-0.569	-1.194

The NETCTL 1.2 was used to generate cytotoxic T-lymphocyte (CTL) epitopes for ULBP2. The server assigns a score to each epitope. This database detects a T-cell epitope that could be identified by CD8+ T cells and activates either deep and exclusive cytotoxic immune responses, and a total of seven 9mer epitopes were collected for the analysis of ULBP2 are shown in table 5.

Table no 5: The prediction of Cytotoxic T-lymphocytes epitopes (CTL)

Epitopes of CTL	Scores Prediction	Antigenicity	Scores of Antigenicity
RADPHSLCY	2.9766	Antigen	0.7455
QVDEKTFLH	0.9959	Non-antigen	0.3145
VDEKTFLHY	0.7966	Non-antigen	−0.1148
LRDIQLENY	0.9821	Antigen	0.9038
YTPKEPLTL	0.8023	Non-antigen	−0.2119
KVVAMSFHY	0.9115	Antigen	0.5890
SSGSWQFSF	0.7513	Non-antigen	0.4468

The Helper T-lymphocytes (HTL), on the other hand, are important in both physiologic and cell-mediated immune responses. As a result, the expression of HLA-II limited CD4+ T-cells is critical for the design and production of new vaccines that trigger CTL activity and successful T-helper induction. The NetMHCIIpan 3.2 server has been used, and favourable epitopes with IC50 values less than 50 nM were taken into account are shown in table 6.

Table no 6: The prediction of antigenic T-helper cell epitopes

HLA	Peptide position	IC-50	Peptide cores	Antigenicity	Allergenicity
GKKLNVTTAWKAQNP	03	22.39	LNVTTAWKA	Antigen	Allergen
LGKKLNVTTAWKAQN	04	20.50	LNVTTAWKA	Antigen	Non-Allergen

The respective protein membrane orientation of tertiary structure of ULBP2 as results shown: Depth/hydrophobic thickness of protein membranes are 0.8 ± 2.2 Å, ΔGtransfer: -2.5 kcal/mol, and angle: 86.± 8.°, and embedded residues: 131 amino acid. The linear epitope antigenic prediction of ULBP2 protein is shown in results: location: 91-110_140-159, epitope: NPVLREVVDILTEQLRDIQL, QFSFDGQIFLLFDSEKRMWT, and scores: 1.000, 0.619 through SVIMTrip Tool. The ULBP2-protein structure pocket regions and cavities with the structural and binding specific regions of protein and nucleic acid were identified through server-GHECOM are results shown in figure 7.

European journal of volunteering and community-based projects Vol.1, No 2; 2021
ISSN: 2724-0592 E-ISSN: 2724-1947
Published by Odv Casa Arcobaleno

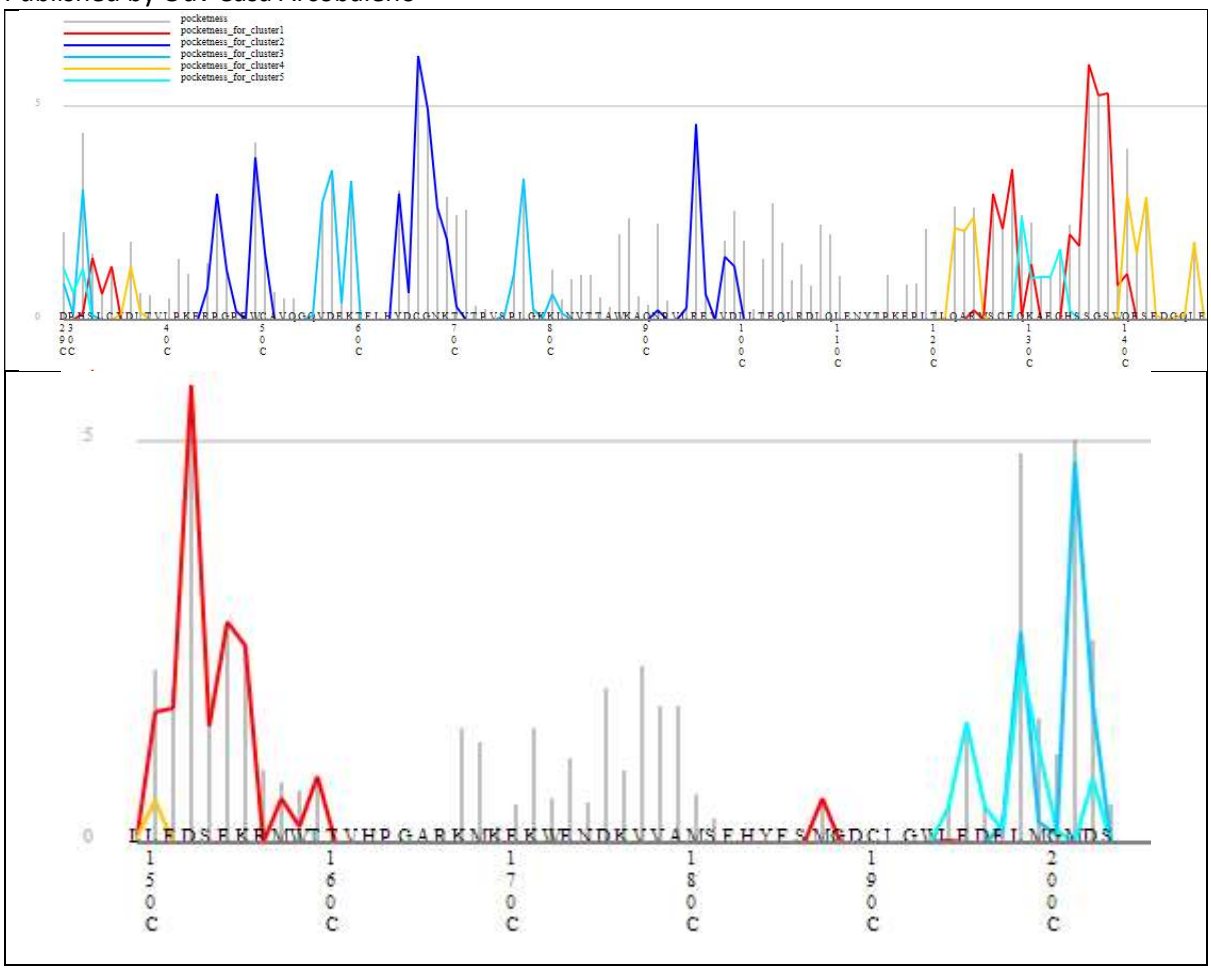
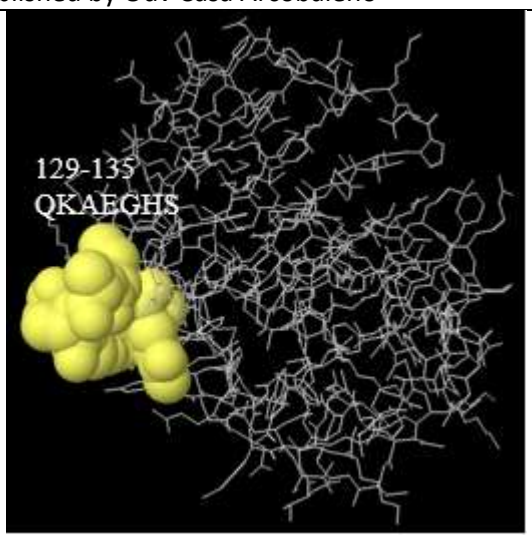
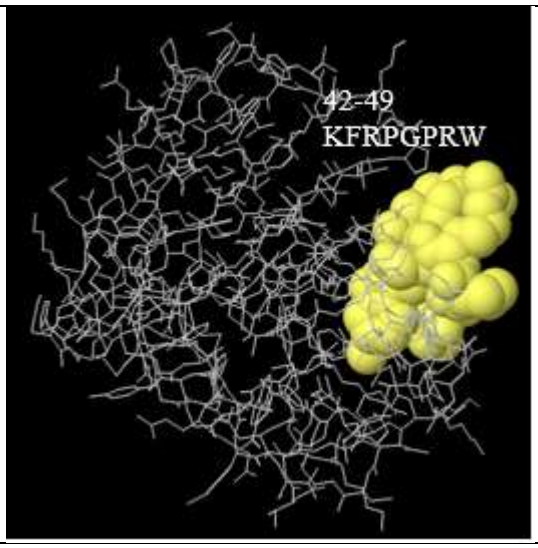


Figure no 7: The pocket regions and cavities of ULBP2 Protein are highlighted in graph with color: **RED**-cluster1, **DARK-BLUE**-cluster2, **DARK-CYAN**-cluster3, **ORANGE**-cluster4, **LIGHT-CYAN**-cluster5, and **Pocketness**: other through GHECOM tool

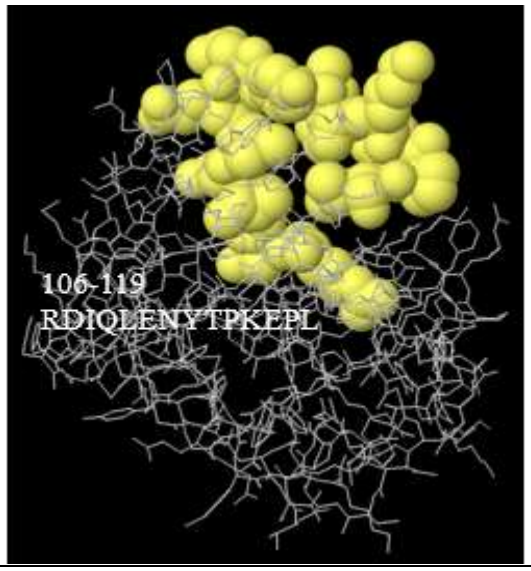
The ULBP2 protein antibodies epitopes result in the range from 129-135, 42-49, 106-119, 66-85, 153-177 peptides: QKAEGHS, KFRPGPRW, RDIQLENYTPKEPL, CGNKTVTPVSPLGKKLNVTT, SEKRMWTTVHPGARKMKEKWENDKV, residues no: 07, 08, 14, 20, 25 with scores: 0.815, 0.769, 0.758, 0.68, 0.638 through ElliPro_IEDB are shown in figure no 8.

A	B

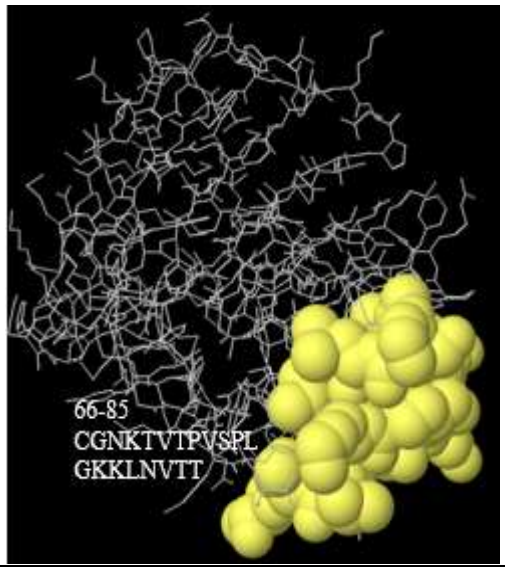

129-135
QKAEGHS


42-49
KFRPGPRW

C


106-119
RDIQLENYTPKEPL

D


66-85
CGNKTVTPVSPL
GKKLNVTT

E

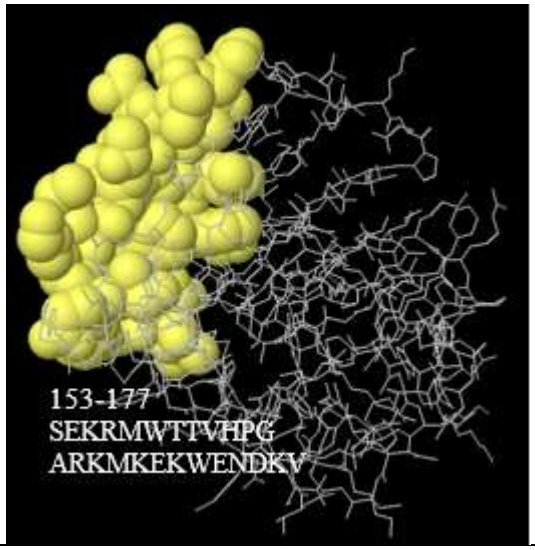

153-177
SEKRMWTTVHPG
ARKMKEKWENDKV

Figure no 8: The ULBP2 protein antibodies epitopes predicted (peptides) results: **A:** QKAEGHS**, B:** KFRPGPRW, **C:** RDIQLENYTPKEPL**, D:** CGNKTVTPVSPLGKKLNVTT**, E:** SEKRMWTTVHPGARKMKEKWENDKV are highlighted in Yellow Domains

4.Discussion

Due to pointlessness of the treatment having many side effects the cancer becomes globally fetal disease. It is caused by alteration in genetic and epigenetic by which the normal cells become cancerous cells. Numerous vaccines are developed but the exact treatment is not introduced because side effects of futility treatments and correct mechanism as well (Kumar, Yadav, Goel, & Rizvi, 2009). To tackle this situation, we have to develop some anticancer agents with less side effects. By using bioinformatics and immune informatics methods ULBP2 protein is used to develop vaccine against cancer (Oyinloye, Adekiya, Aruleba, Ojo, & Ajiboye, 2019).

By using some bioinformatics (Computational) techniques, potential of vaccines and other drugs have been identified. ULBP2 that causes stimulate the B-cells are T-cells can also identify by these computational techniques. In SWISS-MODEL no 3D structures of ULBP2 had stated so we need to used crystal structure of NKG2D with ULBP6 and PDB ID: 4S0U as template. Antigenic epitopes are the portion of proteins having vital information about cancer vaccine because they are present at the surface of antibodies (Aruleba, Adekiya, Oyinloye, & Kappo, 2018; Vangrevelinghe et al., 2003). It is estimated that ULBP2 has eight antigenic peptides having score of 1.0294, with scores above 1.0 which indicate that it is a protein. Vaxijen forecasted is also an antigen having score of 0.5128. Jameson and Wolf stated that higher the antigen leads to higher the antibodies and the B-cells have vital role for antibody development because B-cells epitopes are immunodiagnostics reagents (Sefid, Rasooli, Jahangiri, & Bazmara, 2015). There is total six linear B-cells epitopes (Position 35 coiled, 310 helix, β-strands region; Position 108 was within the coiled β-strands; Position 61 and 159 were in coiled, α-helices and β-strands region; Position 82 was localized in the coiled and α-helices region; while Position 130 was within the coiled and β-strands region). B-cells epitopes are may be continuous and discontinuous or conformational, and they are important for vaccine development (Russell, Parbhoo, & Gildenhuys, 2018; Yao, Zheng, Liang, & Zhang, 2013). By using DiscoTope algorithm tool B cells (conformational and continuous) are joined to form to new large B-cell epitope. LGKKLNVTTAWAQN is a promiscuous epitope because it is non-allergen having IC-50 values below 50 nM. Rather than a molecule made by MHC bounded to the T cells is enough good from the normally T cells because they are not enough good to identify antigens in antigenicity. MHC has 2 classes (Class I and II). Class I is present in nucleated cells and on the other hand class II is present in exogenous antigens. Class I represents endogenous proteins or antigen by cytosolic pathway and class II is present to HTL or CD4+ T cells (Russi, Bourdin, García, & Veaute, 2018). Out of seven epitopes three are antigens, LRDIQLENY makes highest antigen scores means higher affinity to bound with MHC molecules. HTLs are very important for immune system to control immune responses against many diseases (Khatoon, Pandey, & Prajapati, 2017).

Conclusion

From these findings, it can be concluded that, the current bioinformatics techniques are anticipated to have a major impact on cancer therapeutic strategies. As a result, we investigated the molecular and immunogenicity of ULBP2 proteins in order to generate a cancer vaccine. In this present research, the potential epitopes that could be efficient in developing B, and T-

cell mediated immunity in order to obtain the necessary immune response-action in cancer growth is expected. The production of adoptive T-cell and novel tumor drugs transfer holds enormous promise for possible therapeutic drugs. This research-based study might facilitate the against the target of UULBP2 protein against cancer treatment in future on the level of on the epidemiological and genetic characteristics of the population act as novel target.

References

Álvarez, C. A., Gomez, F. A., Mercado, L., Ramírez, R., & Marshall, S. H. (2016). Piscirickettsia salmonis imbalances the innate immune response to succeed in a productive infection in a salmonid cell line model. *PloS one, 11*(10), e0163943.

Aruleba, R. T., Adekiya, T. A., Oyinloye, B. E., & Kappo, A. P. (2018). Structural studies of predicted ligand binding sites and molecular docking analysis of Slc2a4 as a therapeutic target for the treatment of cancer. *International journal of molecular sciences, 19*(2), 386.

Bateman, A., Coin, L., Durbin, R., Finn, R. D., Hollich, V., Griffiths-Jones, S., . . . Sonnhammer, E. L. (2004). The Pfam protein families database. *Nucleic acids research, 32*(suppl_1), D138-D141.

Chen, V. B., Arendall, W. B., Headd, J. J., Keedy, D. A., Immormino, R. M., Kapral, G. J., . . . Richardson, D. C. (2010). MolProbity: all-atom structure validation for macromolecular crystallography. *Acta Crystallographica Section D: Biological Crystallography, 66*(1), 12-21.

DeSantis, C. E., Lin, C. C., Mariotto, A. B., Siegel, R. L., Stein, K. D., Kramer, J. L., . . . Jemal, A. (2014). Cancer treatment and survivorship statistics, 2014. *CA: a cancer journal for clinicians, 64*(4), 252-271.

Dimitrov, I., Bangov, I., Flower, D. R., & Doytchinova, I. (2014). AllerTOP v. 2—a server for in silico prediction of allergens. *Journal of molecular modeling, 20*(6), 1-6.

Doytchinova, I. A., & Flower, D. R. (2007). VaxiJen: a server for prediction of protective antigens, tumour antigens and subunit vaccines. *BMC bioinformatics, 8*(1), 1-7.

EL-Manzalawy, Y., Dobbs, D., & Honavar, V. (2008). Predicting linear B-cell epitopes using string kernels. *Journal of Molecular Recognition: An Interdisciplinary Journal, 21*(4), 243-255.

Fleri, W., Paul, S., Dhanda, S. K., Mahajan, S., Xu, X., Peters, B., & Sette, A. (2017). The immune epitope database and analysis resource in epitope discovery and synthetic vaccine design. *Frontiers in immunology, 8*, 278.

Gasteiger, E., Gattiker, A., Hoogland, C., Ivanyi, I., Appel, R. D., & Bairoch, A. (2003). ExPASy: the proteomics server for in-depth protein knowledge and analysis. *Nucleic acids research, 31*(13), 3784-3788.

Geourjon, C., & Deleage, G. (1995). SOPMA: significant improvements in protein secondary structure prediction by consensus prediction from multiple alignments. *Bioinformatics, 11*(6), 681-684.

Haste Andersen, P., Nielsen, M., & Lund, O. (2006). Prediction of residues in discontinuous B-cell epitopes using protein 3D structures. *Protein Science, 15*(11), 2558-2567.

Khatoon, N., Pandey, R. K., & Prajapati, V. K. (2017). Exploring Leishmania secretory proteins to design B and T cell multi-epitope subunit vaccine using immunoinformatics approach. *Scientific reports, 7*(1), 1-12.

Kumar, B., Yadav, P., Goel, H., & Rizvi, M. (2009). RECENT DEVELOPMENTS IN CANCER THERAPY BY THE USE OF NANOTECHNOLOGY. *Digest Journal of Nanomaterials & Biostructures (DJNB), 4*(1).

Li, K., Mandai, M., Hamanishi, J., Matsumura, N., Suzuki, A., Yagi, H., . . . Konishi, I. (2009). Clinical significance of the NKG2D ligands, MICA/B and ULBP2 in ovarian cancer: high expression of ULBP2 is an indicator of poor prognosis. *Cancer immunology, immunotherapy, 58*(5), 641-652.

Lomize, M. A., Pogozheva, I. D., Joo, H., Mosberg, H. I., & Lomize, A. L. (2012). OPM database and PPM web server: resources for positioning of proteins in membranes. *Nucleic acids research, 40*(D1), D370-D376.

Ma, X., & Yu, H. (2006). Cancer issue: global burden of cancer. *The Yale journal of biology and medicine, 79*(3-4), 85.

Marcus, A., Gowen, B. G., Thompson, T. W., Iannello, A., Ardolino, M., Deng, W., . . . Raulet, D. H. (2014). Recognition of tumors by the innate immune system and natural killer cells. *Advances in immunology, 122*, 91-128.

McGilvray, R. W., Eagle, R. A., Rolland, P., Jafferji, I., Trowsdale, J., & Durrant, L. G. (2010). ULBP2 and RAET1E NKG2D ligands are independent predictors of poor prognosis in ovarian cancer patients. *International journal of cancer, 127*(6), 1412-1420.

Mistry, A. R., & O'Callaghan, C. A. (2007). Regulation of ligands for the activating receptor NKG2D. *Immunology, 121*(4), 439-447.

Oyinloye, B. E., Adekiya, T. A., Aruleba, R. T., Ojo, O. A., & Ajiboye, B. O. (2019). Structure-based docking studies of GLUT4 towards exploring selected phytochemicals from Solanum xanthocarpum as a therapeutic target for the treatment of cancer. *Current drug discovery technologies, 16*(4), 406-416.

Pettersen, E. F., Goddard, T. D., Huang, C. C., Couch, G. S., Greenblatt, D. M., Meng, E. C., & Ferrin, T. E. (2004). UCSF Chimera—a visualization system for exploratory research and analysis. *Journal of computational chemistry, 25*(13), 1605-1612.

Ponomarenko, J., Bui, H.-H., Li, W., Fusseder, N., Bourne, P. E., Sette, A., & Peters, B. (2008). ElliPro: a new structure-based tool for the prediction of antibody epitopes. *BMC bioinformatics, 9*(1), 1-8.

Raulet, D. H., Gasser, S., Gowen, B. G., Deng, W., & Jung, H. (2013). Regulation of ligands for the NKG2D activating receptor. *Annual review of immunology, 31*, 413-441.

Russell, B. L., Parbhoo, N., & Gildenhuys, S. (2018). Analysis of conserved, computationally predicted epitope regions for VP5 and VP7 across three orbiviruses. *Bioinformatics and biology insights, 12*, 1177932218755348.

Russi, R. C., Bourdin, E., García, M. I., & Veaute, C. M. I. (2018). In silico prediction of T- and B-cell epitopes in PmpD: First step towards to the design of a Chlamydia trachomatis vaccine. *biomedical journal, 41*(2), 109-117.

Scholz, E. M., Marcilla, M., Daura, X., Arribas-Layton, D., James, E. A., & Alvarez, I. (2017). Human leukocyte antigen (HLA)-DRB1* 15: 01 and HLA-DRB5* 01: 01 present complementary peptide repertoires. *Frontiers in immunology, 8*, 984.

Schwede, T., Kopp, J., Guex, N., & Peitsch, M. C. (2003). SWISS-MODEL: an automated protein homology-modeling server. *Nucleic acids research, 31*(13), 3381-3385.

Sefid, F., Rasooli, I., Jahangiri, A., & Bazmara, H. (2015). Functional exposed amino acids of BauA as potential immunogen against Acinetobacter baumannii. *Acta biotheoretica, 63*(2), 129-149.

Shulman, L. N., & Mok, T. (2015). Special Issue on Global Cancer Medicine. *Journal of clinical oncology: official journal of the American Society of Clinical Oncology, 34*(1), 1-2.

Szklarczyk, D., Morris, J. H., Cook, H., Kuhn, M., Wyder, S., Simonovic, M., . . . Bork, P. (2016). The STRING database in 2017: quality-controlled protein–protein association networks, made broadly accessible. *Nucleic acids research*, gkw937.

Vangrevelinghe, E., Zimmermann, K., Schoepfer, J., Portmann, R., Fabbro, D., & Furet, P. (2003). Discovery of a potent and selective protein kinase CK2 inhibitor by high-throughput docking. *Journal of medicinal chemistry, 46*(13), 2656-2662.

Wiederstein, M., & Sippl, M. J. (2007). ProSA-web: interactive web service for the recognition of errors in three-dimensional structures of proteins. *Nucleic acids research, 35*(suppl_2), W407-W410.

Yao, B., Zhang, L., Liang, S., & Zhang, C. (2012). SVMTriP: a method to predict antigenic epitopes using support vector machine to integrate tri-peptide similarity and propensity. *PloS one, 7*(9), e45152.

Yao, B., Zheng, D., Liang, S., & Zhang, C. (2013). Conformational B-cell epitope prediction on antigen protein structures: a review of current algorithms and comparison with common binding site prediction methods. *PloS one, 8*(4), e62249.