doi.org/10.36721/PJPS.2022.35.3.REG.731-739.1

In silico-prediction of chloroquine as a multi-targeted drug against CDKN2A signaling network associated with cutaneous malignant melanoma

Zainab Jan[1]*, Muhammad Aqeel[2], Ammara Munir[3,4], Aamir Saeed[1], Haleema Sadia[6], Saeeda Kalsoom[3], Tariq Nadeem[3,5], Zainab Bashir[1], Larayb Awan[1], Sami ud din[7] and Ghulam Muhammad Ali[2]

[1]Department of Bioinformatics, Hazara University, Mansehra, Pakistan
[2]National Institute for Genomics and Advance Biotechnology, NARC, Islamabad, Pakistan
[3]Department of Biotechnology, Virtual University, Pakistan
[4]Department of Biotechnology, University of Azad Jammu and Kashmir, Pakistan
[5]National Center of Excellence in Molecular Biology, University of the Punjab, Lahore, Pakistan
[6]Department of Biotechnology, BUITEMS, Quetta, Pakistan
[7]Department of Genetic, Hazara University Mansehra, Pakistan

Abstract: Melanoma is one of the most common skin infections, has triggered significant morbidity and mortality across the globe. Previous studies have reported that mutations in CDKN2A signalling network is associated with cutaneous malignant melanoma. In the present study, initially, the BioGrid database was utilized, and then hierarchical clustering was performed to identify the CDKN2A signature pathways. In addition, a GO Enrichment analysis was investigated using DAVID (n=187 genes) toolkit. Subsequently, the cBioPortal cancer genomic platform was exploited using alteration ranked frequency to determine the role of the CDKN2A signaling network in 363 samples of cutaneous malignant melanoma patients and we find that CDKN2A and its close interactors PTEN and HUWE1 show highest mutations. Further, we systematically employed molecular docking approach via MOE to target PTEN, CDKN2A and HUWE1 with chloroquine which is naturally occurring in medicinal plant *Nigella sativa* (NS) and observed virtuous interactions between all receptors and ligand molecules with a binding energy of -11.379, -10.324 and -9.06 Kcal/mol, respectively. The outcomes obtained stipulate a vigorous research resource for using chloroquine as a multitargeted anticancer drug. This novel evidence should help the development of effective therapeutic compounds for the treatment of cancer. Our results reveal that chloroquine is a relevant and novel potential therapeutic drug for the treatment of melanoma.

Keywords: Melanoma, chloroquine, signaling network, molecular docking, cancer.

INTRODUCTION

Cancer is the most common cause of death worldwide after cardiovascular disorder (Bray *et al*., 2013). Melanoma is a global tragedy which is causing 75% of deaths that arises from skin cancer (Kilcar *et al*., 2016). Many factors have been reported as the causes of melanoma. Out of these, environmental and genetic risk factors mostly provide a platform for its origin. Exposure to ultraviolet radiation (UVR) is the main environmental risk factor for melanoma (Fortes *et al*., 2016). In the United States, 87,110 new cases and 9730 deaths cases were reported during 2017 (Gangadhar *et al*., 2017; Kang *et al*., 2021).

Approximately, 5% to 10% of all melanomas are hereditary and 40% are caused by alterations in the territory of cyclin-dependent kinase inhibitor 2a (CDKN2A) gene. The chromosome 9p21 resides CDKN2A usually translates two proteins named p16INK4/ and p14ARF and two exons 1α and 1β numbered 2 and 3 are potent for encoding these proteins, respectively. The proteins p16INK4/and p14ARF regulate the G1 phase in combination with cyclin-dependent kinase 4 CDK4 of cell cycle-related to apoptotic phenomenon (Fontana *et al*., 2019). The melanoma penetrance for CDKN2A carriers increases with age and varies between geographical areas. Bishop and his colleagues stated that in careers there were high percentages of melanoma penetrance up to 13 to 50% in Europe, Australia, and the United States at child ages while in later ages even at 80 in similar regions; it ranges from 58 to 91% (Harland *et al*., 2014). Recently, about 48 p16INK4a alterations reported in more than 100 melanoma-susceptible histories of family worldwide. The high-level association between a polymorphism in the genomic region of p16INK4A and melanoma suggests that functional defects identification is clinically essential to explore cancer-related alterations. The American Cancer Society justified the prevention practices including to avoid exposure to the sun apply sunscreen in bigger volume, proper covering dress along with

**Corresponding author:* e-mail: zainab.bioinfo@gmail.com

sunglasses. Recently few novel studies confirmed the mutations of CDKN2A that performed vital role to exploit the risk correlated with melanoma origin by analysing the whole CDS territories (Schrom *et al*., 2020; Bruno *et al*., 2016).

Chloroquine (CHQ) is a famous antimalarial extracted from *Nigella sative* (NS) drug that has recently attracted scientist and researchers due to its anti-neoplastic activities. Drug re-purposing is a famous protocol for investigating new therapeutic application for drugs that have been initially designed for various medical benefits. Drug re-purposing is also a useful technique for the identification of application of chloroquine for cancer treatment (Touret *et al*., 2021).

In the current scenario, we employed an *in-silico* approaches to investigate the effect of chloroquine as an anti-cancer drug. First, we explored the mutations in CDKN2A signaling network associated with melanoma skin cancer then its expression at the whole network level was analysed to find out therapeutic targets. Finally, these targets were docked with chloroquine for drug designing and development.

MATERIALS AND METHODS

Detailed protocol employed to complete this study was illustrated in fig. 1.

Network Analysis

To understand the CDKN2A interaction network, the graphically representation of the network was retrieved from the BIOGRID database (www.biogrid.org) which was a large collection of protein-protein interaction networks, and their interactors were mainly of physical and genetic modes. The network included all neighbours of the CDKN2A gene based on ranked alteration frequency implemented. After entering query sequences, we got interactors and network of CDKN2A. Edges and nodes both were color-coded which represented the interactors and the frequency of genes were highlighted. The query genes of CDKN2A were represented with a dark colour and their interactors were distributing around them (Jan *et al*., 2021).

Enrichment Analysis and Hubs genes identifications

Enrichment analysis was performed in the DAVID (www.david.ncifcrf.gov) toolkit which is a large collection of functional annotation cluster and annotation chart (Jan *et al*., 2018). Function discovery associated with large dataset is an important prediction which covers biological information assigned in genomics. The Database for Annotation, Visualization, and Integrated Discovery (DAVID) v6.7 tool was exploited for functional enrichment through curated dictionary of functional terms and in this way, we were able to predict their gene ontology functions and KEGG annotation terms. During this procedure, high enrichment scores were taken into account, controlling false discovery rates FDR < 0.1 by following the method reported by Benjamini and Hochberg (Benjamini *et al*., 1995).

By using DAVID, we determined the participation of interactors in different diseases. All the interactors were clustered using hierarchical clustering method then followed by fisher exact test. The interactor's enriched terms were selected based on their significance at ($P< 0.05$) by rejecting the null hypothesis. Double screening method was employed to identified Hub genes in CDKN2A network using HUBBA analyser.

Cancer Genomes Mutation Summary

Irrevocably, we employed cBioPortal (www.cbioportal.org) to retrieve the large-scale cancer genomics data sets associated with CDKN2A and its interactors. cBioPortal is an interactive opensource cancer genome platform provides a web-based resource for processing of multidimensional dataset in a user-friendly way and is used for exploring large-scale cancer genomic data sets. The cBioPortal can execute genomic alterations in the form of mutations, copy-number alterations (CNAs), z-score based differential levels of mRNA and miRNA and makes graphics using cancer gene sets, considers the co-ordinations among genes, polymorphisms in these genes across the samples. We employed ranked alteration frequency to screen alterations in CDKN2A and its interactors residing in TCGA using cutaneous melanoma as a base. For the targeted gene set, the name of interactors was entered as a query. In cbioportal, firstly, we select cancer study “cutaneous melanoma (TCGA, PanCancer atlas)” then data type priority “Mutation and CNA.”

Genetic polymorphism summary

An Oncoprint was elucidated to summarize the polymorphisms in CDKN2A and its interactors by using cancer-related samples. In the oncograph, rows represented genes and columns represented the samples. Genetic alterations including mutation, deletion, and amplification based on ranked frequency using Onco Query Language were summarized by color-coding.

Co-expression analysis

For co-expression analysis, Spearman and Pearson methods were employed to find the threshold p-value less than 0.05 by rejecting the null hypothesis. Different interactors showed different p values with different genes in cutaneous melanoma (Kumari *et al*., 2012).

Molecular Docking studies

The 3D structure of CDKN2A, PTEN and HUWE1 molecules were collected from PDB and saved in PDB format while 2D structure of chloroquine was obtained

from PubChem saved in SDF format. Ligand and Protein was prepared for docking studies. The ligand format was changed using ChemDraw and save as mol format with aim to open these files in MOE after structure preparation (Cousins *et al*., 2011). The ligand structure was protonating 3D at a temperature of 310 °C and pH 7.00 and energy minimized using MOE2015, with default parameters. The MMFF94× force field was employed without any periodicity while the constraints were preserved at the rigid water molecule level. To conduct molecular docking, the receptors were exposed, polar hydrogens were added, however on the other hand the ligand atom was chosen and rescoring1 was set on London dG and rescoring2 on GBVI/WSA dG (Rahman *et al*., 2019). Finally, molecular docking was running to illustrate the ligand interaction with protein. Protein-ligand docking score, ligand properties, and 2D and 3D structures were kept for assessment.

STATISTICAL ANALYSIS

All the statistical analysis were performed using R 4.0.5 to show the statistical significant of the study.

RESULTS

Signaling network of CDKN2A

CDKN2A signaling network retrieved from the BioGRID database. The CDKN2A showed 309 physical interactions and 3 genetic interactions. The overall signaling network statistics showed that it consists of a total of 184 nodes and 585 edges. Out of 184 nodes, the 171 nodes showed affiliations to same organisms while 12 nodes showed with different organisms. From 585 edges the 573 are= physical edges, 3 genetic edges and nine physical/genetic edges. The CDKN2A does not contain any chemical edges (fig. 2).

Hub Genes Identifications

Top three hub nodes, identified in the present study, have been strongly associated with melanoma skin cancer, including PTEN, CDKN2A and HUWE1. Hub nodes are defined as the genes which are extremely associated with other genes, and they were anticipated to have significant functions in biological process and pathways development. Meanwhile hub nodes have more multifaceted interactions as compared to other genes, they might have fundamental contribution in the underlying mechanisms of disease (Zhai *et al*., 2019). However, hub genes identification involved in development and progression of melanoma possibly will lead to the development of better therapeutic agents (fig. 3).

Oncogenomic study

For the complete analysis of the network components involved in the selected melanoma, we used cBioPortal a cancer genome platform that provides a huge collection of cancer datasets with attractive graphical presentation and comprehensive oncogene detection with accurate ratio and mutation site. In the present study, OncoPrint, co-expression and mutations were analyzed. A dataset of 363 samples of cutaneous melanoma (TCGA, PanCancer atlas) was taken in which 363 samples showed alterations of which 20 cases showed amplification, 6 cases showed deep deletion, 185 cases showed multiple alterations and 134 cases showed the mutation. The overall study showed a 95.04% alteration in which there were 5.51% amplification, 1.65% deletion, 50.96% multiple alteration and 36.91% mutation. In our study we identified PTEN, HUEW1 and CDKN2A show abundant mutations (fig. 4).

CDKN2A signaling network interactor's enrichment analysis

Functional annotation of CDKN2A signaling network interactors was analysed using the DAVID toolkit. It showed that the network interactors are involved in different biological process and molecular functions, which help to understand the whole network involvement in disease. Functional annotation clustering showed the biological process in which disease enrichment analysis lies under the categories of functional annotation chart. The biological process table listed below showed CDKN2A signalling network play an important role in the different biological process such as DNA replication, Transcription, Cell cycle, cell division, etc (table 1). CDKN2A network components, showed the participation of interactors in different diseases like cancer, tumor, hepatitis, salmonella infection, and bladder cancer, etc. (table 2).

Co-expression Analysis

Integration between pathways, related to morphological variations, and the transcripts is conferred by a powerful tool such as co-expression analyses (Ferreira *et al*., 2016; Chandran *et al*., 2016). Coexpression analyses of some interactors were performed by Spearman and Pearson methods. The co-expression analysis showed that events in CDKN2A versus CDKN2A-DT were likely to cooccur in cutaneous melanoma (p=0.03). The Pearson correlation coefficient for CDKN2A versus CDKN2A-AT is 0.78 while Spearman correlation coefficient is 0.79. Some genes lie on the same cytoband while other genes lie on different cytobands. Mostly interactors and their correlated gene have p-value equal to 0.03 while the highest p coefficient value is p=0.09. The lowest p coefficient value is 0.01 (table 3).

Mutual exclusivity and co-occurrence summary

Mutual exclusivity showed rough connection between different genes through statistical approach. In mutually exclusive, gene-related events related with a precise cancer are frequently mutually exclusive in a cluster of tumors-explicitly, only one genetic event is probable to happen in each cancer sample, while the another

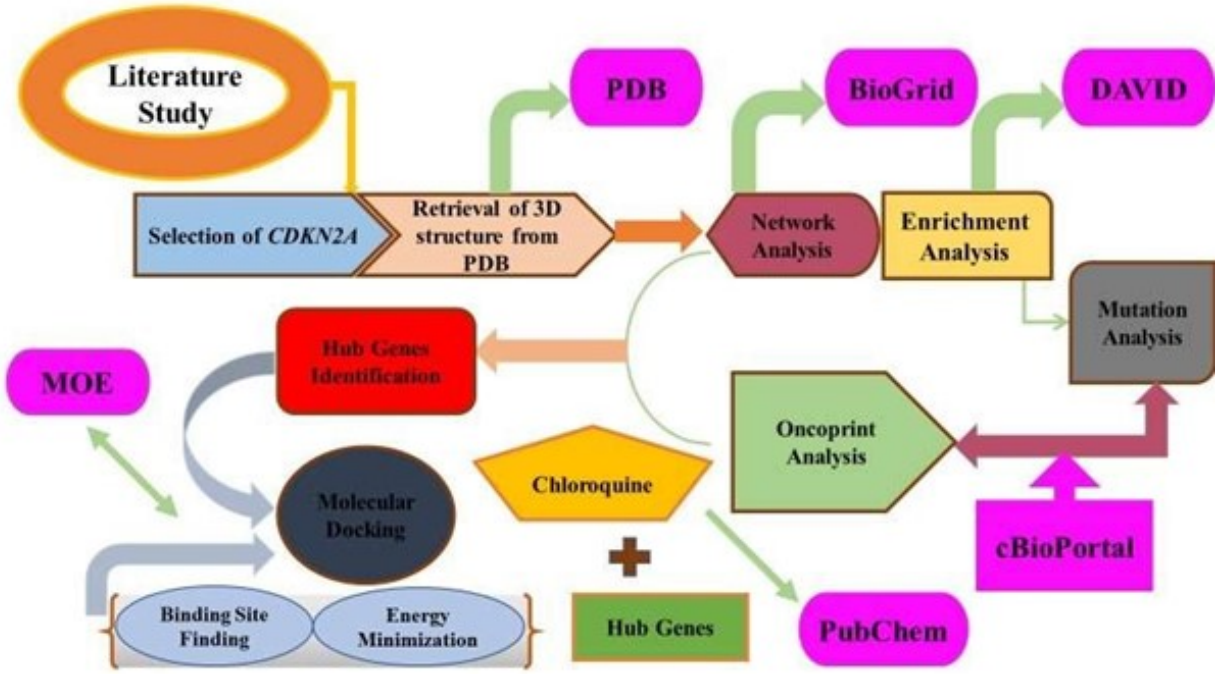

Fig. 1: Complete Study Protocol

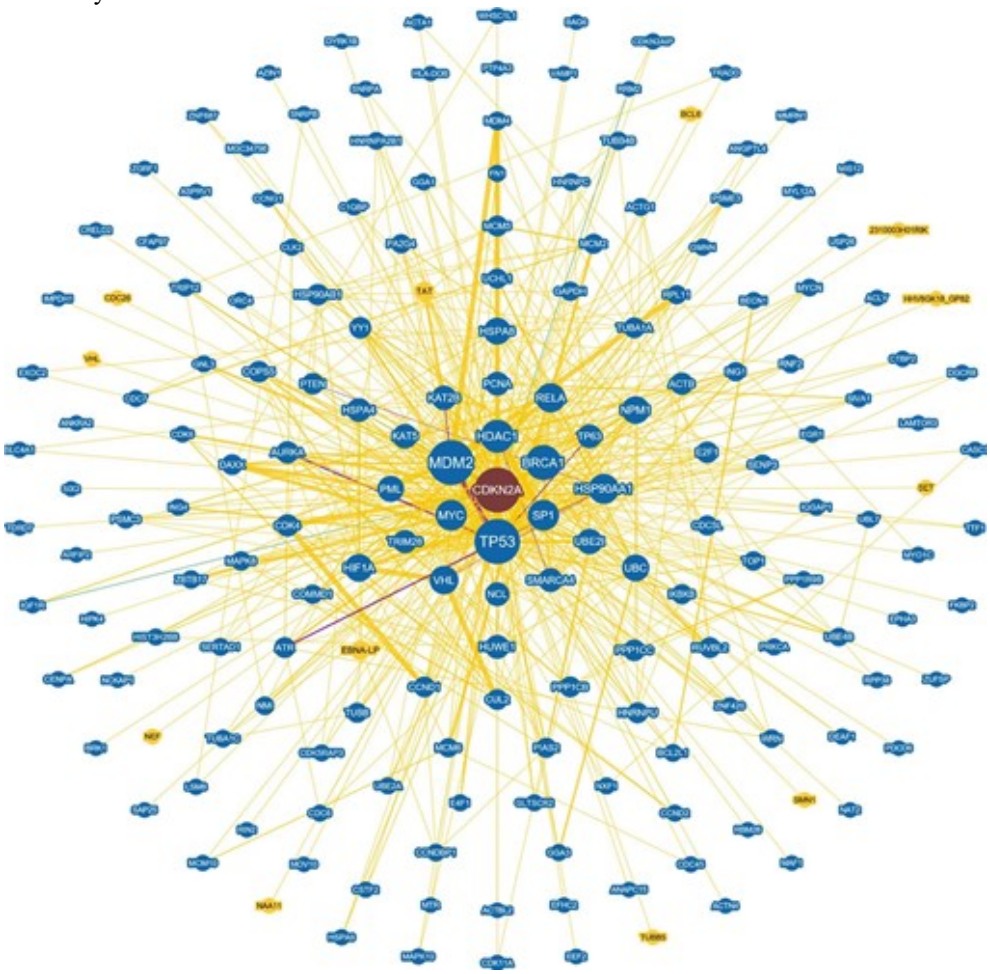

Fig. 2: CDK2NA signalling network

Table 1: Function annotation clustering of CDKN2A network from DAVID

S. NO	Biological process	No. of Proteins
1	DNA replication	8
2	Transcription	40
3	G1/S transition of mitotic cell cycle	13
4	P53 signalling pathway	10
5	Protein phosphorylation	11
6	Protein binding	156
7	Pathways in cancer	24
8	Cell cycle	11
9	Nucleotide binding	45
10	Regulation of signal transduction by p53 class mediator	12
11	S-nitrosylation	8
12	Cell Division	18
13	Mitosis	9
14	ATP-binding	39
15	mRNA processing	12

Table 2: Function annotation chart for disease enrichment analysis

S. NO	Disease enrichment analysis	No of Proteins
1	Pancreatic cancer	10
2	Hepatitis B	12
3	Influenza A	11
4	Tumor	7
5	Colorectal cancer	5
6	Mental retardation	10
7	Thyroid cancer	3
8	Salmonella infection	5
9	Bladder cancer	5

Table 3: Co-expression Analysis of CDKN2A and its interactors.

Gene	Correlated Gene (G)	Cytoband of Gene	Cytoband of (G)	p-value	Pearson Correlation coefficient	Spearman Correlation coefficient
CDKN2A	CDKN2A-DT	9p21.3	9p21.3	0.03	0.78	0.79
HUWE1	EDA2R	12q15	Xp15	0.03	0.61	0.70
CDK4	METTL1	12q14.1	12q14.1	0.03	0.81	0.70
CDK6	BACH1	7q21.2	21q21.3	0.04	0.71	0.67
TP63	CSTA	3q28	3q21.1	0.04	0.58	0.66
TP53	GEMIN4	17p13.1	17p13.1	0.05	0.43	0.54
BRCA1	TOP2A	17q21.31	17q21.2	0.04	0.73	0.67
CCND1	CTTN	11q13.3	11q13.3	0.08	0.45	0.40
GGA3	UNK	17q25.1	17q25.1	0.04	0.68	0.63
FN1	LOXL2	2q35	8p21.3	0.06	0.51	0.49
PTEN	PTENP1	10q23.31	9q13.3	0.01	0.94	0.93

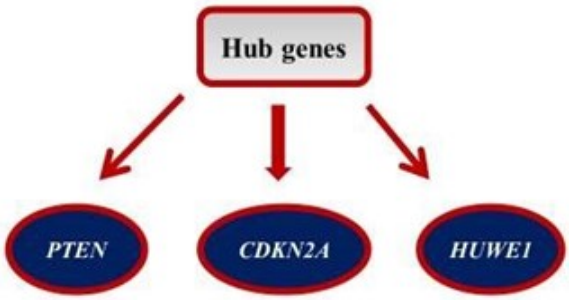

Fig. 3: Hub genes identification using Hubb Analyzer

Table 4: Significant association table (FDR > 0.05)

S. No	Association counter parts	P-Values	Q-Values	Odd Ratio
1	PTP4A3 and AZIN1	0.001	0.001	3
2	HLA-DOB and BAG6	0.001	0.001	3
3	MDM2 and CDK4	0.001	0.002	3
4	MDM2 and CDK4	0.001	0.002	3
5	BAG6 and MYO1C	0.001	0.006	3
6	HSP90AB1 and USP26	0.001	0.012	3
7	MCM2 and UBE4B	0.001	0.013	3
8	MCM6 and TP63	0.001	0.022	2.791
9	CDKN2A and CDK4	0.022	0.022	-1.369
10	UBE4B and CDK11A	0.001	0.025	3
11	BAG6 and IQGAP1	0.004	0.047	2.201

Table 5: Non-significant association table (FDR > 0.05)

S. No	Association counter parts	P-Values	Q-Values	Odd Ratio
1	ZNF420 and MAPK8	0.015	0.692	2.959
2	SERTAD1 and CTBP2	0.004	0.174	3
3	HSPA9 and CDKN2AIP	0.008	0.174	3
4	SLC4A1 and TRDD7	0.005	0.174	2.381
5	E2F1 and MAPK10	0.023	0.407	2.706
6	ZBTB17 and E4F1	0.013	0.59	3
7	BRAC1 and HDAC1	0.077	0.59	2.534
8	PA2G4 and ZUP1	0.038	0.59	3
9	C1QBP and NCKAP1	0.019	0.727	3
10	ZGRF1 and KAT2B	0.003	0.603	2.489
11	EEF2 and DYRK1B	0.008	0.296	3
12	ATR and IKBKB	0.01	0.437	1.958
13	SP1 and UCHL1	0.002	0.094	3
14	RIN2 and SNRPA	0.002	0.057	3
15	AURKA and HSP90AA1	0.043	0.805	3

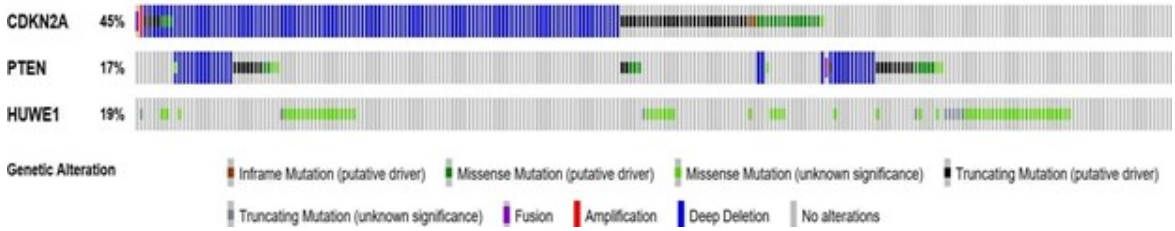

Fig. 4: Genomic alteration summary of hub genes involved in cutaneous malignant melanoma

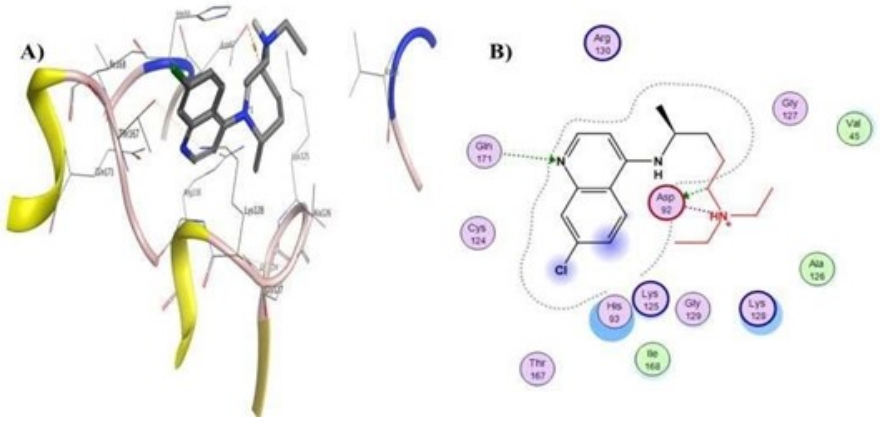

Fig. 5: Interaction analysis of PTEN with chloroquine

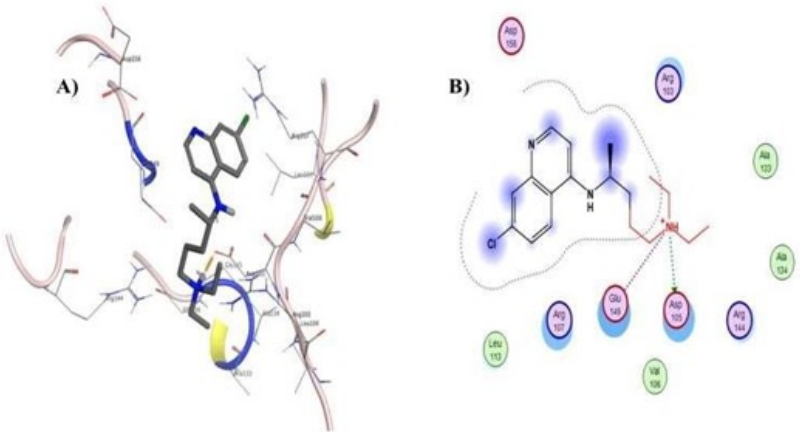

Fig. 6: Interaction analysis of CDKN2A with chloroquine

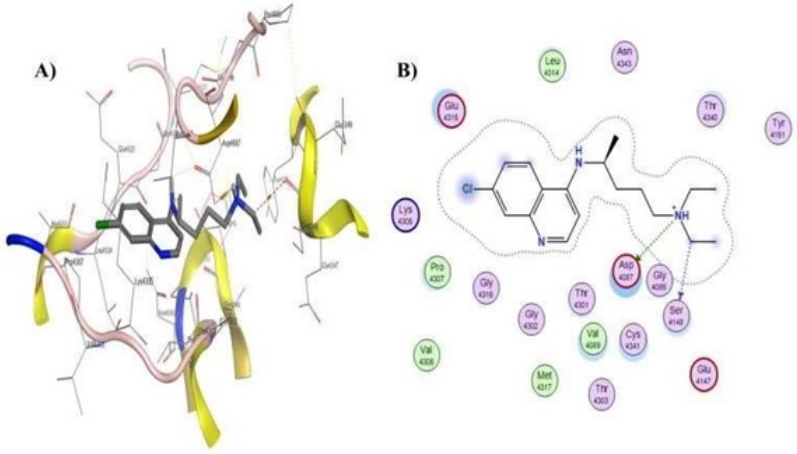

Fig. 7: Interaction studies of HUEW1 gene with chloroquine

condition is the co-occurrence which mean several genes are altered in the identical cancer sample. Mutual exclusivity showed the genes that alter signaling in subcutaneous melanoma. That exclusive property summarizes the statistical analyses related to the significant associativity of PTP4A3 and AZIN1 pair (FDR < 0.05). Here all mutual association pairs have been shortlisted according to their level of significance. Among these, strongest association was observed in PTP4A3 and AZIN1 pair (p-value = 0.001, q-value = 0.001 and odd $\log_2$ ratio = 3) as in table 3. Similarly, two pairs HLA-DOB and BAG6 (p-value = 0.001, q-value = 0.001 and odd $\log_2$ ratio = 3); and MDM2 and CDK4 (p-value = 0.001, q-value = 0.002 and odd $\log_2$ ratio = 3) are on the second and spot based on strongest association in terms of relationships with melanoma tab provides summary statistics on mutual exclusivity and co-occurrence of genomic alterations in each pair of query genes. In this example, all three pairs have a tendency toward mutual exclusivity. Although the PTP4A3-AZIN1 pair has the strongest tendency toward mutual exclusivity, the relationship is not statistically significant (P = 0.11). The mutual exclusivity is significant for the other two gene pairs (P<0.05, red outline). The P values are determined by a Fisher's exact test with the null hypothesis that the frequency of occurrence of a pair of alterations in two genes is proportional to their uncorrelated occurrence in each gene. While the rest of insignificance cases are given in table 4 & 5.

Molecular docking studies of CDKN2A, HUWE1, PTEN with Chloroquine

Molecular docking techniques is commonly utilized for the identification of binding orientation between small molecules and drug candidates to their protein targets and to predict their binding affinity and activity (Lee *et al*., 2019). MOE was employed to dock CDKN2A, HUWE1, PTEN with chloroquine. The docking studies implies that HUWE1 showed good binding affinity with chloroquine followed by CDKN2A while PTEN showed least binding affinity. For PTEN, we found that only one residue showed interaction with ligand that is Gln171, it formed hydrogen bond with chloroquine with docking score of -11.379. Although for CDKN2A, we detect that Glu149 form ionic bond with chloroquine while Asp105 formed hydrogen bond with a docking score of -10.324. Similarly, HUWE1 showed good interaction with ligand under a docking score of -7.06. For HUWE1 gene,

ASP4087 formed hydrogen bond with ligand while Ser14148 formed covalent bond with ligand. While all other residues of receptor's molecules formed nonbinding contacts with ligand molecule. From protein-ligand docking survey, we deduced that CDKN2A has strong binding connection with chloroquine, although it is weaker than HUWE1 and strong than PTEN. It is speculated that chloroquine can be used as an antimelanoma drug. But there are no published articles that are directly related to anti-melanoma activity of chloroquine. According to the most recent research paper on the anticancer effect of chloroquine along with 5-Fluorouracil (5-FU) against human colon cell, report that chloroquine along with 5-FU is a potential and effective candidate combination therapy along with 5-FU for the treatment of colorectal cancer. This docking result indicates chloroquine is a suitable candidate for treatment of skin infections (figs. 5 & 6 &7).

DISCUSSION

Melanoma is one of the most severe types of skin cancer, with rapid metastasis, progression, and a significant risk of death, especially when discovered late (Stueven *et al*., 2017 and Umar *et al*., 2020). Resistance occurs through malignant heterogeneity, alternative pathways, and some substantial adverse circumstances, limiting the efficacy of novel treatments, even though a significant number of medications have recently been created for late-stage melanoma cancer (Marra *et al*., 2018). Numerous studies have reported a mutation in the CDKN2A network led to the development of cutaneous melanoma.

In present work, BioGrid was used identify the CDKN2A network and to find the therapeutic targets then dock the targets with Chloroquine. Following that, the cBioPortal cancer genomic platform was employed to find the role of the CDKN2A signalling network in 363 patients with cutaneous malignant melanoma using alteration ranking frequency, and we discovered that CDKN2A and its near interactors PTEN and HUWE1 have the most mutations.

Over expression of these hub genes promotes abnormality in function and disturbs cell cycle, which can cause abnormal cell division, genetic instability, cellular proliferation, and tumorigenesis. The regulatory process of normal cell division and growth is known as the cell cycle that underwent four basic phases G1, S, G2 and M phase. CDKN2A is a gene that encodes protein p16, which regulate cell cycle and inhibits the activities of two kinases protein cdk4 and cdk6 that can phosphorylate the retinoblastoma protein (RB) in the G1 phase of cell cycle (Jan *et al*., 2021; Sherr *et al*., 1999). In the present study, we demonstrated the potentiation inhibitory effect of chloroquine as an anticancer agent against melanoma using bioinformatics approach. The CDKN2A signaling network was retrieved to understand the molecular mechanism of CDKN2A and its interactors. Enrichment analysis was performed to achieve the functional understanding. Oncogenomic studies was carried out to perform mutational analysis, oncoprint, co-expression and genetic polymorphism analysis. From double screening method, we deduce that PTEN and HUWE1 are hubs genes showing high contribution in malignant melanoma. Impulsively, a wide range of mutations were observed on various domains of CDKN2A, PTEN and HUWE1.

Molecular docking is the most extensively utilized approach for predicting protein-ligand interactions. The MOE software was used to analyse the binding modes and binding interaction. The results show that chloroquine which is present in *Nigella sative* (NS) has a high binding affinity for PTEN (-11.379 kcal/mol) followed by CDKN2A (-10.324 kcal/mol) and HUWEI (-9.06 kcal/mol). Combining the results in this study with the network analysis coupled with molecular docking we identified that the chloroquine has an anticancer activity and good inhibitory effect for CDKN2A, PTEN and HUWEI.

CONCLUSION

In this research, we clearly demonstrated that multitargeted therapy is an effective and promising strategy for the treatment of cutaneous malignant melanoma. Bringing in mind, we obtained CDKN2A signaling network from BioGrid to identify most significant targets. The expression level of these significant interactors was determined by enrichment analysis. Double screening approach was used to identify hub genes. The resulted 3 genes were docked with chloroquine using MOE as a docking platform. In the future, these 3 genes could be used as a significant target for the treatment of melanoma and chloroquine can be used as an anticancer drug. Also, our study showed as a source of pointers for CDKN2A network-based drug designing and multi-targeted therapy. It will be important for deep understanding to find out the cause of cutaneous malignant melanoma and to devise a strategy to detect and reverse this mutation by CRISPR or other genome editing techniques.

ACKNOWLEDGMENTS

We thank to the faculty of department of bioinformatics, Hazara university mansehra Pakistan and NIGAB, Narc Islamabad, Pakistan.

REFERENCES

Benjamini Y and Hochberg Y (1995). Controlling the false discovery rate: A practical and powerful approach to multiple testing. *J. R. Stat*, **57**(1): 289-300.

Bray F, Ren JS, Masuyer E and Ferlay J (2013). Global estimates of cancer prevalence for 27 sites in the adult population in 2008. *Int. J. Cancer Res.*, **132**(5): 1133-1145.

Bruno W, Pastorino L, Ghiorzo P, Andreotti V, Martinuzzi C, Menin C, Elefanti L, Stagni C, Vecchiato A, Rodolfo M and Maurichi A (2016). Multiple primary melanomas (MPMs) and criteria for genetic assessment: MultiMEL, a multicenter study of the Italian Melanoma Intergroup. *JAAD*, **74**(2): 325-332.

Chandran AKN, Jeong HY, Jung KH and Lee C (2016). Development of functional modules based on co-expression patterns for cell-wall biosynthesis related genes in rice. *J. Plant Biol.*, **59**(1): 1-15.

Ferreira SS, Hotta CT, De Carli Poelking VG, Leite DCC, Buckeridge MS, Loureiro ME, Barbosa MHP, Carneiro MS and Souza GM (2016). Co-expression network analysis reveals transcription factors associated to cell wall biosynthesis in sugarcane. *Plant. Mol. Biol.*, **91**(1-2): 15-35.

Fontana R, Ranieri M, La Mantia G and Vivo M (2019). Dual role of the alternative reading frame ARF protein in cancer. *Biomolecules*, **9**(3): 87.

Fortes C, Mastroeni S, Segatto MM, Hohmann C, Miligi L, Bakos L and Bonamigo R (2016). Occupational exposure to pesticides with occupational sun exposure increases the risk for cutaneous melanoma. *J. Occup. Environ*, **58**(4): 370-375.

Gangadhar TC, Hwu WJ, Postow MA, Hamid O, Daud A, Dronca R, Joseph R, O'Day SJ, Hodi FS, Pavlick AC and Kluger H (2017). Efficacy and safety of pembrolizumab in patients enrolled in KEYNOTE-030 in the United States: an expanded access program. *J. Immunother (Hagerstown, Md.,* **40**(9): 334.

Harland M, Cust AE, Badenas C, Chang YM, Holland EA, Aguilera P, Aitken JF, Armstrong BK, Barrett JH, Carrera C and Chan M (2014). Prevalence and predictors of germline CDKN2A mutations for melanoma cases from Australia, Spain and the United Kingdom. *Hered Cancer Clin. Pract.,* **12**(1): 1-10.

Jan Z, Khan MS, Ahmad SU, Saadia H, Ullah I, Said A, Ullah H, Khan MK, Khan H and Khan MK (2021). Evaluation of cyclin-dependent kinase inhibitor signalling network in esophageal adenocarcinoma Via computational and statistical approaches. *Bio. Res*., **18**(3): 2022-2029.

Jan Z, Bashir Z and Aqeel M (2018). Identification of genetic alteration of TP53 gene in uterine carcinosarcoma through oncogenomic approach. *PJCIS*, **3**(2), 41-46.

Kang AS and Lee DJ (2021). Melanoma Risk Factors and Prevention. *In*: Practical Manual for Dermatologic and Surgical Melanoma Management. Springer, Cham., Pp.15-27.

Kilcar AY, Tekin V, Muftuler FZB and Medine EI (2016). 99m Tc labeled plumbagin: Estrogen receptor dependent examination against breast cancer cells and comparison with PLGA encapsulated form. *J. Radioanal. Nucl. Chem*, **308**(1): 13-22.

Kumari S, Nie J, Chen HS, Ma H, Stewart R, Li X, Lu MZ, Taylor WM and Wei H (2012). Evaluation of gene association methods for co expression network construction and biological knowledge discovery. *PloS One*, **7**(11): e50411.

Lee ACL, Harris JL, Khanna KK and Hong JH (2019). A comprehensive review on current advances in peptide drug development and design. *Int. J. Mol. Sci.*, **20**(10): 2383.

Marra A, Ferrone CR, Fusciello C, Scognamiglio G, Ferrone S, Pepe S and Sabbatino F (2018). Translational research in cutaneous melanoma: New therapeutic perspectives. *Curr. Med. Chem*, **18**(2): 166-181.

Rahman N, Muhammad I, Khan H, Aschner M, Filosa R and Daglia M (2019). Molecular docking of isolated alkaloids for possible α-glucosidase inhibition. *Biomolecules*, **9**(10): 544.

Schrom Kory P, InYoung Kim and Elma D Baron (2020). The immune system and pathogenesis of melanoma and non-melanoma skin cancer. *In*: Sunlight, Vitamin D and Skin Cancer, Springer, Cham. pp.211-226.

Sherr CJ and Roberts JM (1999). CDK inhibitors: positive and negative regulators of G1-phase progression. *Genes Dev.*, **13**(12): 1501-1512.

Stueven NA, Schlaeger NM, Monte AP, Hwang SPL and Huang CC (2017). A novel stilbene-like compound that inhibits melanoma growth by regulating melanocyte differentiation and proliferation. *Toxicol. Appl. Pharmacol*, **337**: 30-38.

Touret F and de Lamballerie X (2020). Of chloroquine and COVID-19. *Antivir. Res*, **177**: 104762.

Umar AB, Uzairu A, Shallangwa GA and Uba S (2020). QSAR modelling and molecular docking studies for anti-cancer compounds against melanoma cell line SK-MEL-2. *Heliyon*, **6**(3): e03640.

Zhai T, Muhanhali D, Jia X, Wu Z, Cai Z and Ling Y (2019). Identification of gene co-expression modules and hub genes associated with lymph node metastasis of papillary thyroid cancer. *Endocrine*, **66**(3): 573-584.