Molecular Docking of Onco-mirs expressed in HCC with novel anti-cancer protein LHPP

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ABSTRACT

Hepatocellular carcinoma is sixth major widespread cancer and the third major reason of global death caused by different types of cancer. It is crucial cause for cancer deaths among almost 80% of total liver cancers. The miRNAs are non-coding endogenous, single-chain, small RNA of approx 20-24 nucleotides having role in post-transcriptional regulation of gene expressed in the organisms by influencing either stability or translational step of mRNAs. LHPP (Phospholysine Phosphohistidine Inorganic pyrophosphate Phosphatase) is a new class of inorganic pyrophosphatase, believed to be crucial for life and having role in the tumor suppression. Here in current research work a successful attempt has been made for the docking of ligand LHPP, an anti-cancerous protein with the earlier prepared molecular models of miRNAs such as miR-21, 155, 221 & miR-224. Docking of all four selected template miRNAs & ligand molecule LHPP was performed separately by the RNA-Protein docking server HDock, Docking score of best model i.e. model-1 for miR-21 is -298.13, for mi-R-155 is -291.00 for miR-221 is -309.65 and for miR-224 is -286.13. Docking score, confidence score and RMSD value obtained for all four micro-RNAs are showing the good quality docking with LHPP ligand molecule.

KEYWORDS: Micro-RNA, LHPP, Docking, Anti-cancer molecule

INTRODUCTION

Liver cancer is the crucial cause for cancer deaths among almost 80% of total liver cancers and its global incidence varied to a great extent with geographic variations i.e. highest approx 81% cases occurred in developing countries (Dasgupta et al 2020; Llovet et al 2021). Hepatocellular carcinoma is sixth major widespread cancer and the third major reason of global death caused by different types of cancer (Sung et al 2021). The miRNAs are non-

coding endogenous, single-chain, small RNA of approx 20-24 nucleotides having role in post-transcriptional regulation of gene expressed in the organisms by influencing either stability or translational step of mRNAs (Rhim et al 2022; Liu et al 2018; Ambros et al 2003). These have major negative regulatory action by binding to 3' UTR (un-translated region) on target mRNA (Wang et al 2022). Generally miRNAs have role in array of cellular tasks in a complex manner such as proliferation of cells, immune system related responses, responses in DNA damage, tumor development and inhibition of apoptosis (Bartel 2004). The miRNAs are released in the circulation either passively from dead cells or actively by micro-vesicles & exosomes which are comparatively stable inside circulation (Mitchell et al 2008).

As per current reports, approx 2000 types of miRNAs have been recorded in human cells & have their role in regulation of approx 30% of genome (Khare et al 2022) There are several reports ensures the physiological correlation between liver cancer development and its prognosis. Particularly the microRNAs miR-21, 221, and miR-222 has been establish over-expressed in liver cancer patient in compare to normal livers (Karakastanis et al 2013). The miR-21 is the foremost discovered oncogenic micro RNA i.e. onco-miR, have role in cell cycle regulation and cancer genesis (Zhang et al 2020). It might be served as an early stage prognostic potential biomarker for hepatocellular carcinoma (Demerdash et al 2017). Different research result data suggested that expression pattern of miR-21 was reported constantly as upregulated trend in the diagnosis of patient with liver diseases in comparison to healthy people, signifying it could be regard as a potential diagnostic and therapeutic moleculees (Bautista-Sánchez et al 2020; Wagennar et al 2015). The miR-155 is a micro RNA which has role in inflammation and works as a regulator of immune responses through pro-inflammatory activities (Hu et al 2002). So it could be a potential factor in the correlation of inflammation-mediated cancer genesis. Furthermore, as evident by research findings, miR-155 has a role in the prognosis prediction and progression of HCC (Zhang et al 2020). As per the research results, it's found that there is noteworthy higher expression level of miR-155 in liver cancer patient in compare to healthy control, promoting the fact that it could be used as a potential biomarker for liver cancer (Ratnasari et al 2022). The miR-221 expression was found upregulated in HCC and expression involved in the cancer genesis and progression (He et al 2014). It is observed that expression of miR-221 was normally higher in liver cancer sample in comparison to healthy sample and also it promotes the ability to growth of liver cells with carcinogenic infection (Rong et al 2013; Yun et al 2022). Research studies suggest that miR-221 is concerned in HCC initiation, invasion, and metastasis, so it could be served as a potential gene target for HCC treatment (Liu et al 2016). Dysregulation of miR-224 has

been established in different types of malignancies in the human and primarily act as onco-miR in breast cancer, thyroid cancer etc (Zang et al 2020; Wang et al 2008). The miR-224 is an onco-miR and expression was observed upregulated in HCC patients and it's involved in promotion of HCC cell proliferation (Miao et al 2020). Along with it has role in the earlier tumor progression and metastasis (Meng et al 2007). The miR-224 could be used as promising early-stage prognostic biomarker for HCC detection (Yang et al 2020).

LHPP (Phospholysine Phosphohistidine Inorganic pyrophosphate Phosphatase) is a new class of inorganic pyrophosphatase and is believed to be crucial for life (Klumpp et al 2002). Gene encoding LHPP is located on chromosome 10 q26.13, containing 3 domain of leucine zipper and expressed universally in liver, kidney and brain (Yokoi et al 2003). It is a phosphatase and its primary action is removal of phosphate groups linked with histidine from proteins. If there is absence of LHPP then histidine phosphorylation of global protein will increases and it activates cell proliferation in uncontrolled manner, which is finally leads to formation of tumor (Hindupur et al 2018). However, interaction of LHPP with protein have significant role in poor prognosis of tumor as well as it’s proliferation and invasion (Matthews et al 1995). LHPP works as a tumor suppressor protein was first time experimentally proved in 2018 by a group of scientist (Hindupur et al 2018) and from that to current scenario research has been conducted on different type of cancer such as liver, oral, pancreatic & cervical carcinoma etc. to investigate their anti-cancer effects (Liu et al 2022; Wu et al 2020; Zheng et al 2018) Several research results suggested that LHPP is an important tumor suppressor protein which have role in the inhibition of all stages of hepatocellular carcinoma *viz* cell proliferation, cell growth and metastasis (Ma et al 2022; Liao et al 2022; Chao et al 2021). Furthermore, the decreased expression of LHPP is being associated with AFP, so LHPP might be act as a promising potent prognostic factor in HCC diagnosis (Chao et al 2021).

Here in current research work a successful attempt has been made for the docking of ligand LHPP, an anti-cancerous protein with the earlier prepared molecular models of miRNAs such as miR-21, 155, 221 & miR-224.

MATERIALS & METHODS

Anti-cancer protein LHPP 3D structure

First of all 3-D crystal structure of human LHPP was downloaded from PDB database and PDB ID is 2X4D. Structure was downloaded in PDB format, which is the required format for the docking software. 3-D structure of LHPP used for the further docking was submitted to PDB database by Vollmar *et al.*, in 2010. Downloaded structure was prepared by X-Ray diffraction method with resolution of 1.92 Å, which represent the good quality of structure.

Molecular docking using HDOCK Server

HDOCK server has incorporation bundle of several components which includes algorithm for docking and scoring purposes, various third-party programs, and a group of tools prepared by self server. Molecular docking method performed by HDOCK server is simple & fully automated i.e. there are several inbuilt tools & programmes which are operated in background to smoothen the docking process and results are given by the server in few times as a webpage.

Input of data is first step of HDOCK programme which may be either sequences as well as structures. Input data in structure format is propping up for DNAs and RNAs presently, confront to modeling DNA and RNA structures using selected sequences. The second step involves search based on sequence similarity. Third step is identification of available common records to detect same PDB codes between ring two given set of templates. The 3-D models were prepared by MODELLER while sequence alignment was accomplished by ClustalW for templates selected. Finally global docking was performed for each structures either uploaded by server or any users. Hierarchical FFTbased docking program HDOCKlite developed to global sample presumed binding orientations.

RESULTS & DISCUSSION

Docking of template miRNAs miR-21, 155, 221 & miR-224 with ligand LHPP

Docking of all four selected template miRNAs & ligand molecule LHPP was performed separately by the RNA-Protein docking server HDock, which provide top-10 docking model for each miRNAs such as Model-1 to Model-10 in PDB format on the basis of rank 1-10. Model-1 is the best docking model for each mi-RNAs and LHPP generated through HDock server, which is ranked 1st on the basis of docking score value provided by the server for all 10 models of each miRNAs. The values for confidence score and ligand RMSD (Å) were also provided by the server for all 10 models of each miRNAs, along with docking score values.

Docking details of miR-21 with LHPP

For miR-21 docking model, best docking model i.e. Model-1 is represented in Figure 1 while overlapped representations of top 10 docking models generated between the interaction of miR-21 and ligand LHPP are depicted in Figure 2. Docking score of best model i.e. model-1 is -298.13 while the docking score of rest 9 models are less negative than model-1. Details of all 10 predicted models for miR-21 with anticancer protein LHPP with details of docking score, confidence score and ligand RMSD are mentioned in Table 1. The details of each residues of best model (i.e. model-1) involved in the interaction between receptor and ligand interface in docking of miR-21 with LHPP are given in Supplementary Table 1.

Docking details of miR-155 with LHPP

For miR-155 docking model, best docking model i.e. Model-1 is represented in Figure 3 while overlapped representations of top 10 docking models generated between the interaction of miR-155 and ligand LHPP are depicted in Figure 4. Docking score of best model i.e. model-1 is -291.00 while the docking score of rest 9 models are less negative than model-1. Details of all 10 predicted models for miR-21 with anticancer protein LHPP with details of docking score, confidence score and ligand RMSD are mentioned in Table 2. The details of each residues of best model (i.e. model-1) involved in the interaction between receptor and ligand interface in docking of miR-155 with LHPP are given in Supplementary Table 2.

Docking details of miR-221 with LHPP

For miR-221 docking model, best docking model i.e. Model-1 is represented in Figure 5 while overlapped representations of top 10 docking models generated between the interaction of miR-221 and ligand LHPP are depicted in Figure 6. Docking score of best model i.e. model-1 is -309.65 while the docking score of rest 9 models are less negative than model-1. Details of all 10 predicted models for miR-221 with anticancer protein LHPP with details of docking score, confidence score and ligand RMSD are mentioned in Table 3. The details of each residues of best model (i.e. model-1) involved in the interaction between receptor and ligand interface in docking of miR-221 with LHPP are given in Supplementary Table 3.

Docking details of miR-224 with LHPP

For miR-224 docking model, best docking model i.e. Model-1 is represented in Figure 7 while overlapped representations of top 10 docking models generated between the interaction

of miR-224 and ligand LHPP are depicted in Figure 8. Docking score of best model i.e. model-1 is -286.13 while the docking score of rest 9 models are less negative than model-1. Details of all 10 predicted models for miR-224 with anticancer protein LHPP with details of docking score, confidence score and ligand RMSD are mentioned in Table 4. The details of each residues of best model (i.e. model-1) involved in the interaction between receptor and ligand interface in docking of miR-224 with LHPP are given in Supplementary Table 4.

CONCLUSION

Structural and functional understandings of different miRNAs have developed quickly in the last few years. Molecular modeling of structures by the vigilant assessment of nucleotide sequences by the use of *in silico* approach provides fundamentals for bioinformatics. However, *in silico* molecular docking study has initial advantage in context of simplicity and time saving ability in comparison to *in vitro* approach.

The current study intends to perform the molecular docking study with earlier prepared structure of four most important miRNAs articulated in hepatocellular carcinoma regulation using *in silico* method. All four selected micro-RNAs miR-21, miR-155, miR-221 & mir-224 are onco-mir in nature i.e. all are upregulated in the cancer development. LHPP is an established anti-cancer protein which is selected as ligand molecule for the molecule docking study with all four onco-mirs. Selection of LHPP as a ligand molecule in the current study has 2 benefits, firstly LHPP works as anti-cancer protein, so this molecule always show effects in down regulation of the cancer development process. Secondly, there is good binding affinity and good docking score between LHPP and all four onco-mirs such as micro-RNAs miR-21, 155, 221 & miR-224 evident by the molecular docking experiment performed, so it must binds with onco-mirs in great extent. Furthermore, due to binding of oncomirs with LHPP less number of onco-mirs will be available to express inside the cell. Finally the expression of onco-mirs are become downregulated and ultimately responsible for the inhibition of the cancer development and progression. On the basis of aforesaid experiment LHPP would be a good molecule for the regulation of Hepatocellular carcinoma based on the micro-RNAs.

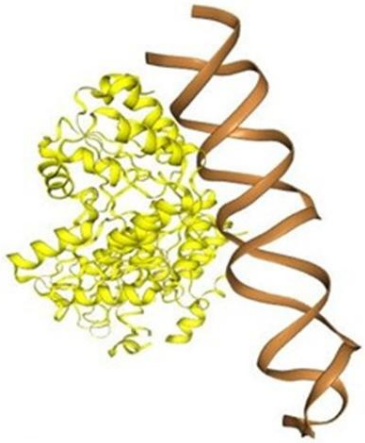

Figure 1: Best docked model of miR-21 with anticancer protein LHPP

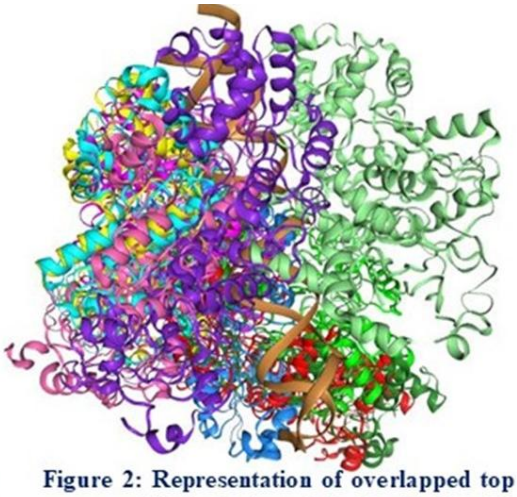

Figure 2: Representation of overlapped top 10 docked model of miR-21 with anticancer protein LHPP

Figure 7: Best docked model of miR-224 with anticancer protein LHPP

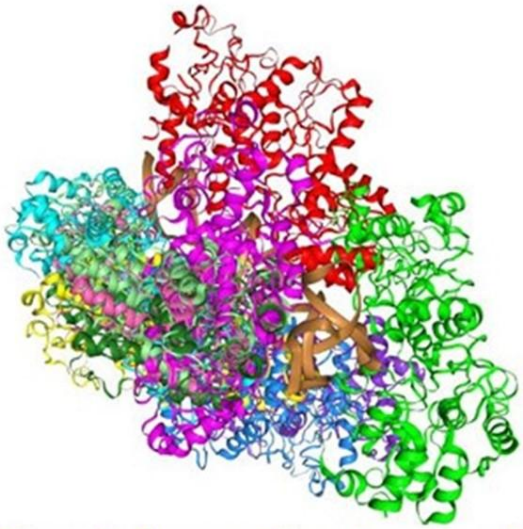

Figure 8: Representation of overlapped top 10 docked model of miR-224 with anticancer protein LHPP

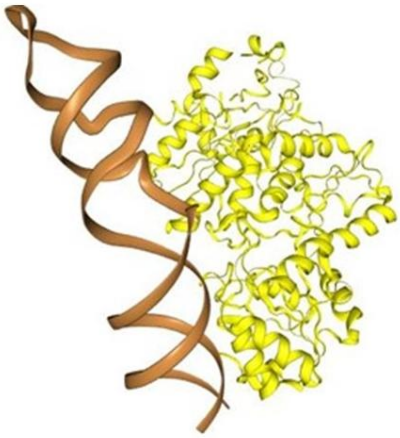

Figure 3: Best docked model of miR-155 with anticancer protein LHPP

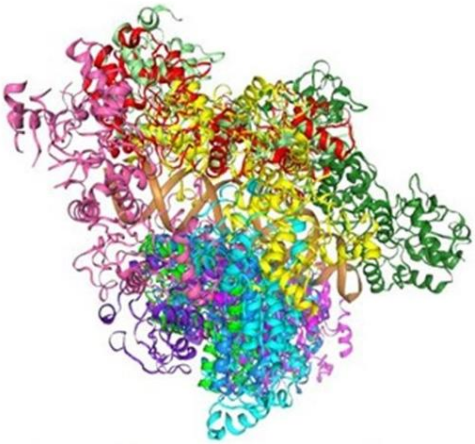

Figure 4: Representation of overlapped top 10 docked model of miR-155 with anticancer protein LHPP

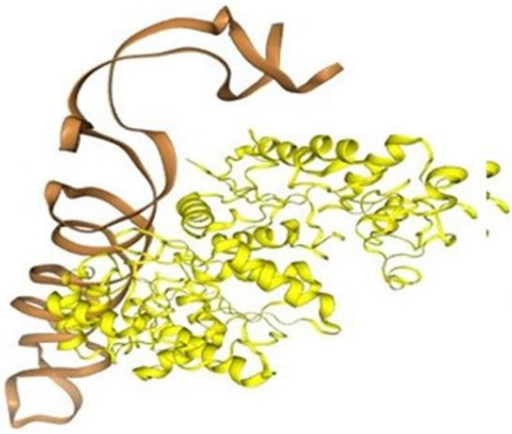

Figure 5: Best docked model of miR-221 with anticancer protein LHPP

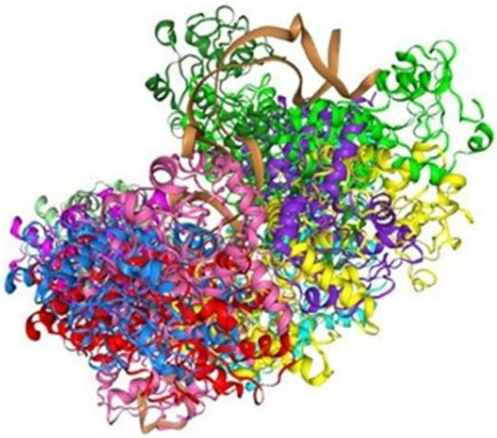

Figure 6: Representation of overlapped top 10 docked model of miR-221 with anticancer protein LHPP

Table 1: Summary of Top 10 docked models for miR-21 & LHPP

Rank (Model)	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Docking Score	-298.13	-277.46	-270.87	-269.08	-267.27	-260.08	-259.27	-257.71	-252.67	-252.20
Confidence Score	0.9509	0.9275	0.9181	0.9154	0.9126	0.9004	0.8989	0.8961	0.8863	0.8853
Ligand RMSD (Å)	122.84	136.82	153.38	116.52	124.52	136.20	154.41	141.62	144.62	131.25

Table 2: Summary of Top 10 models for miR-155 & LHPP

Rank (Model)	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Docking Score	-291.00	-268.61	-266.95	-261.22	-260.08	-255.59	-252.10	-251.89	-250.94	-248.09

Confidence Score	0.9437	0.9147	0.9121	0.9024	0.9004	0.8920	0.8851	0.8847	0.8828	0.8767
Ligand RMSD (Å)	100.64	146.95	90.15	159.44	137.47	147.01	131.69	138.40	91.08	98.25

Table 3: Summary of Top 10 models for miR-221 & LHPP

Rank (Model)	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Docking Score	-309.65	-302.57	-299.16	-296.97	-296.22	-293.38	-290.66	-284.19	-282.27	-281.20
Confidence Score	0.9606	0.9548	0.9518	0.9498	0.9490	0.9462	0.9434	0.9361	0.9337	0.9324
Ligand RMSD (Å)	137.45	105.37	141.58	108.49	110.15	139.24	97.29	115.11	101.08	141.47

Table 4: Summary of Top 10 models for miR-224 & LHPP

Rank (Model)	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Docking Score	-286.13	-282.73	-280.18	-279.02	-278.84	-276.55	-268.41	-268.12	-267.14	-267.07
Confidence Score	0.9383	0.9343	0.9311	0.9296	0.9294	0.9263	0.9144	0.9139	0.9124	0.9122
Ligand RMSD (Å)	126.88	174.03	126.09	144.34	121.63	144.40	131.90	143.93	128.97	129.18

Author Contribution

NK and NS has conceptualized the work and designed initial Methodology. NK and AT have conducted laboratory analysis. AS and NS have analyzed results. All authors have contributed toward compilation of the manuscript.

Conflict of interest

Authors declare that there exist no conflict of interest.

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