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An In-Silico Approach for Analysing the interplay of Hepatitis B viral X protein with Human Adaptin protein.

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ABSTRACT: Hepatitis B virus (HBV) is the predominant cause for the liver-related malignancies worldwide. Recently, clathrin mediated pathway has been found to assist the Hepatitis B virion in entering the host cell successfully. The role of the viral L protein subunit (LHB) has been understood to interact with clathrin proteins during the clathrin mediated endocytosis. The role of the viral X protein is necessitated here owing to the regulatory nature of this protein during the pathogenesis, which however has been quite elusive. Therefore, an in-silico study has been designed to assess the interaction possibilities between the adaptor protein Gamma 2 adaptin and the regulatory viral protein X (HBX). This study is designed to predict the interaction between Viral HBx protein and human Gamma 2 adaptin protein of clathrin mediated endocytic pathway during the Hepatitis B viral infection using insilico protein protein docking tools. The protein complexes Gamma-2-Adaptin-HBx and Gamma-2-Adaptin-LHB were then docked using Cluspro, Hex and HDock respectively. We found that Gamma-2-Adaptin-HBx binding energies using Cluspro and hex we more negative as compared to the ones obtained for the Gamma-2-Adaptin-LHB complex. This study indicates towards the HBX viral protein in being on of the important interactors of Gamma 2 adaptin in addition to LHB.

KEYWORDS: Hepatitis B virus; HBV; Viral Protein; Clatherin; Endocytosis; Human.

1. INTRODUCTION

Hepatitis B Virus (HBV) pertains to the Hepadnaviridae family. It is known to infect hepatocytes of humans and some non-human primates. Hepatitis B virus has seven proteins viz: S, C, P and X. The S protein is divided into three subunits: Large (L), Middle (M) and small (S) [1]. All the viral proteins have their own segregated functions during the various stages of viral entry, propagation and pathogenesis. The foremost requirement of HBV for a successful viral infection is to enter its target host cell. HBV enters its target host cell (hepatocytes) by interacting with the heparan sulphate proteoglycans present on the surface of the hepatocytes through endocytosis [2]. This attachment facilitates the viral preS1 protein subunit to form more potent and irreversible interaction with the bile salt receptor which is also known as the sodium taurocholate cotransporting polypeptide (NTCP) [3]. The attachment of the viral protein to the host NTCP receptor recruits another host cell surface protein E-cadherin. The activation of E-cadherin thus causes a change in the cell membrane polarization which thereby promotes the internalization of the viral particle through endocytosis [4].

The relevance of clathrin mediated endocytosis has been recognized recently for HBV [5]. During the infection, the clathrin mediated endocytosis is regulated by certain endosome associated Rab GTPases. Rab5A as well as Rab7A have been found to be recruited by the virus during pathogenesis [6]. Another study describing the pathway involved in endocytosis of epidermal growth factor receptors has been found to be involved in the entry of the virus [7]. This process involved the phosphorylation of epidermal growth factor receptor which subsequently recruited the adaptor molecules such as AP2A1 as well as Eps 15 [8].

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The adaptor proteins or adaptins are one of the most integral components of the clathrin mediated endocytic pathway. There are varied functions which are regulated by various AP complexes in mammals responsible for regulation and coordination of varied functions. For instance, AP1 regulates the transport of lysosomal hydrolases amongst the Trans Golgi Network and endosomes [9]. AP2 associates with the plasma membrane and governs endocytosis pathway [10]. AP3 governs protein trafficking to organelles such as lysosomes. However, the role of AP4 is least understood. Adaptins can be segregated into four classes of molecules i.e. alpha, beta-, beta prime- and gamma- adaptins. Medium and small subunits associated to adaptins constitute a heterotetrameric complex called an adaptor complex, which promotes the formation of clathrin-coated vesicles [11]. In spite the knowledge regarding the functions of adaptins, their role during HBV pathogenesis is scarcely understood. A group of researchers led by Stühler et. al. in 2001 established the physical interaction between the preS1 domain of L protein (LHB) of the virus with Gamma 2 adaptin using yeast two hybrid system and validated using the chromatography and co-immunoprecipitation [12].

Gamma 2 adaptin or the Golgi-localizing, Gamma-adaptin ear domain homology, ARF-binding proteins or simply GGAs pertain to a family of monomeric clathrin adaptor proteins that are conserved from yeasts to humans [13]. GGAs support the clathrin-mediated transport of proteins such as from the Trans Golgi Network to endosomes as well as lysosomes through their interactions with TGN-sorting receptors. Further the study suggested the role of Gamma 2 adaptin in the process of viral biogenesis and pathogenesis mediated by preS1 domain of the L protein hence proving the physiological relevance of this interaction [14].

Based on this study it is interesting to note that this study has not been extended to any other viral protein. During the pathogenesis, there is another viral protein which regulates the host viral protein protein interaction at the various stages of viral progression. This protein is the HBV X protein or HBx. X protein of Hepatitis B virus (HBx) is a non-structural regulatory protein. During pathogenesis, the precise role of this protein is still elusive, nonetheless there are multiple pathways where this protein has been found to be involved. Therefore, the presence of this protein during this stage of pathogenesis cannot be ruled out. Hence, we have designed an *In-silico* based study where we shall compare the binding energies of the complex of Gamma 2 adaptin with HBx complex with the complex of Gamma 2 adaptin with LHB.

Therefore, keeping the functional relevance of HBx protein in view, we are hypothesizing that HBx protein could be involved in the clathrin mediated endocytosis and would interact with the Gamma 2 adaptin to regulate the progression of pathogenesis through its own interaction.

2. RESULTS AND DISCUSSION

2.1. STRING

The interactions PPI network obtained in STRING generated ten known functional associations shown as nodes connected by edges (Figure 1). A more refined network was generated by changing the parameter setting to high confidence score, functional and physical interaction and experimental and co-occurance data (Figure 2) [15]. This gave us the most well studies experimentally three adaptor proteins which seemed to be involved in clathrin-mediated endocytosis. The three associated proteins AP1G2, AP1M2 and AP1S2 were subjected to the further investigation by studying all the experimental evidences provided by the database. We shortlisted AP1G2 adaptor protein due to its exclusivity as an interactor with the large subunit (LHB) of the HBV S protein during the HBV infection. (Figure 1 and Figure 2).

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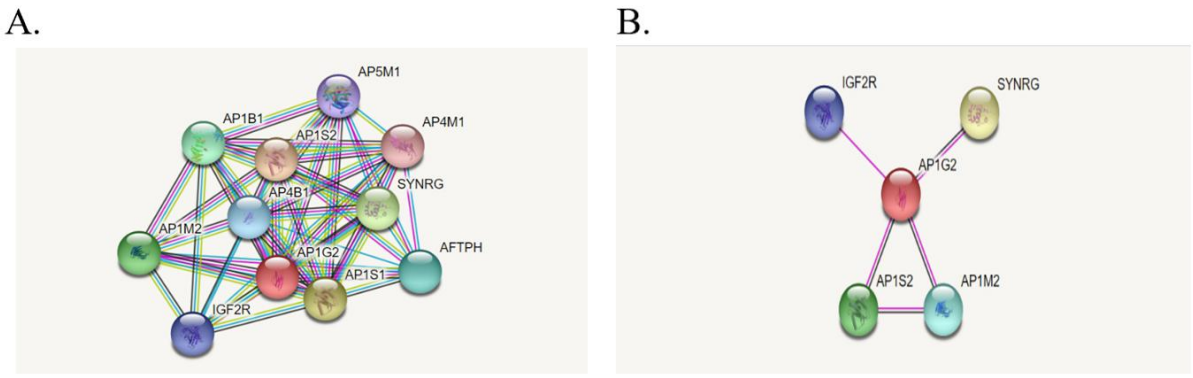

Figure 1: The STRING database was used to extract all the interactors between HBV S protein and Homo Sapiens. The network shown in the Figure (A) represents all the adjoining functional and physical interactions of the direct interactor of HBV S protein i.e. AP-1 complex subunit gamma-like 2 or Gamma 2 Adaptin protein. (B) represents AP1G2 as one of the physical interactor which has been experimentally validated. AP1G2 is AP-1 complex subunit gamma-like 2 which is alternatively known as Gamma 2 adaptin.

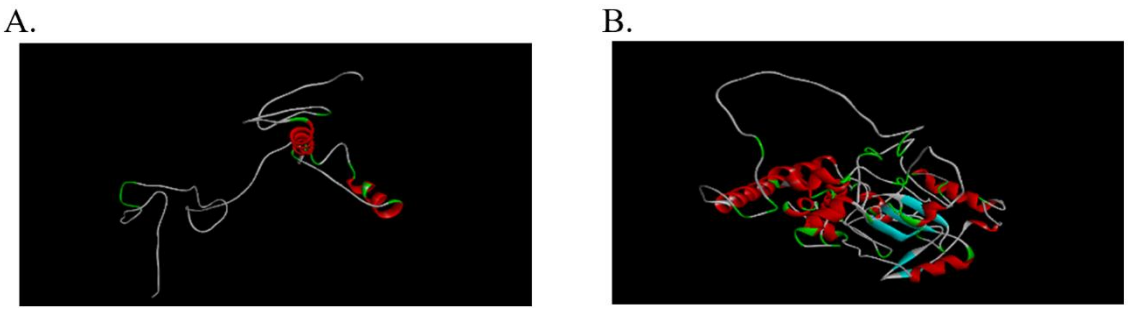

Figure 2: The PORTER 4.0 generated 3-D models of the secondary structures predicted for the viral protein A. Hepatitis B virus X protein and B. Hepatitis B virus Large subunit of S protein. In the figure the beta sheet is represented in blue color and the alpha helix is represented in green and red colors.

2.2 Protein 2D structures

The PORTER 4.0 generated 2-D models of the secondary structures predicted for the viral protein A. Hepatitis B virus X protein and B. Hepatitis B virus Large subunit of S protein. In the Figure 2, the beta sheet is represented in blue color and the alpha helix is represented in green and red colors.

2.3. Protein 3D structures

The I-TASSER generated 3-D models of the viral protein A. Hepatitis B virus X protein and B. Hepatitis B virus Large subunit of S protein. In the Figure 3, the beta sheet is represented in blue color and the alpha helix is represented in green and red colors.

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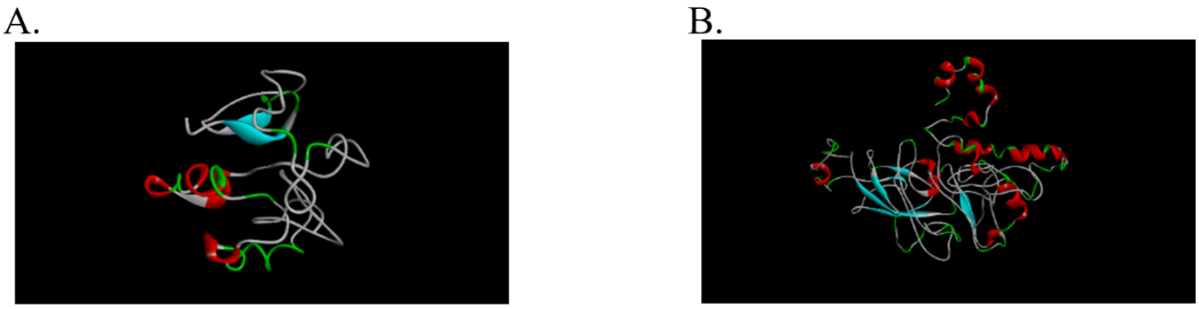

Figure 3: The I-TASSER generated 3-D models of the viral protein A. Hepatitis B virus X protein and B. Hepatitis B virus Large subunit of S protein. In the figure the beta sheet is represented in blue color and the alpha helix is represented in green and red colors.

2.4. Protein model validation

Table 1 represents the Ramachandran plot analysis data of the predicted 3-D structures of the host and viral proteins.

Table 1: Ramachandran plot analysis data: All the proteins considered in the study exhibited a good degree of fold and falling under the acceptable region of protein secondary structure (helix and sheet). Ranging from 90.0% to 100.00% in allowed region and 0.0% to 10.0% amino acid residues falling under the disallowed regions table.

Sr no	Protein name	Chain and Number of residues	Allowed region %	Disallowed region %
1	Gamma Adaptin2	120	100	0.0
2	Gamma X	276	94.4	5.6
3	L Protein	389	96.7	3.3
4	Result Gamma L	511	97.7	2.3
5	X protein	154	90.0	10.0

All the proteins considered in the study exhibited a good degree of fold and falling under the acceptable region of protein secondary structure (helix and sheet). Ranging from 90.0% to 100.00% in allowed region and 0.0% to 10.0% amino acid residues falling under the disallowed regions table.

2.5. Host-viral protein-protein docking

The protein protein interaction was carried out using the most widely used protein protein interaction docking tools. These tools work of varied principles to dock the two interacting proteins and ideally give out the results in the form of negative energy. The docking tools used are Cluspro, HDOck and Hex.

2.5.1. CLUSPRO

The protein protein docking done using Cluspro demonstrated that the score of binding energy was significant and was greater than the binding energy of the docking with viral L protein. The Table 2 represents the cluster which is the best pose of docking; the members where the lowest possible number of members were in the group; weighted score which represents the highest negative energy obtained for the corresponding pose which corresponds to the corresponding clusters. This was the first evidence that X protein can be a good potential interactor with the host Gamma 2 adaptin. The lowest energy scores of the complex loacted at the 0 cluster of adaptin with X protein is -789 whereas for adaptin with L protein of the virus is -714. The 0 cluster demonstrates the best possible interaction of complex of both the proteins which are present in conformation of producing the highest negative energy. These values show significant interaction for HBx and Adaptin more than the LHB-Adaptin which is the experimentally validated interacting molecule. The first 5 cluster value selection showed that consistently the energy values of the docking of adapin with X protein is more negative than the docking score of the adaptin with L protein. The Lowest energy scores of 0 cluster of adaptin with X protein is -789 whereas for adaptin with L protein of the

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virus is -714. The first 5 poses selection showed that consistently the energy values of the docking of adapin with X protein is more negative than the docking score of the adaptin with L protein. Cluster scores were obtained from this equation, generated by the server.

E Balanced = 0.40Erep + −0.40Eatt+600Eelec+1.00EDARS

E Electrostatic favoured = 0.40Erep + − 0.40Eatt + 1200Eelec +1.00EDARS

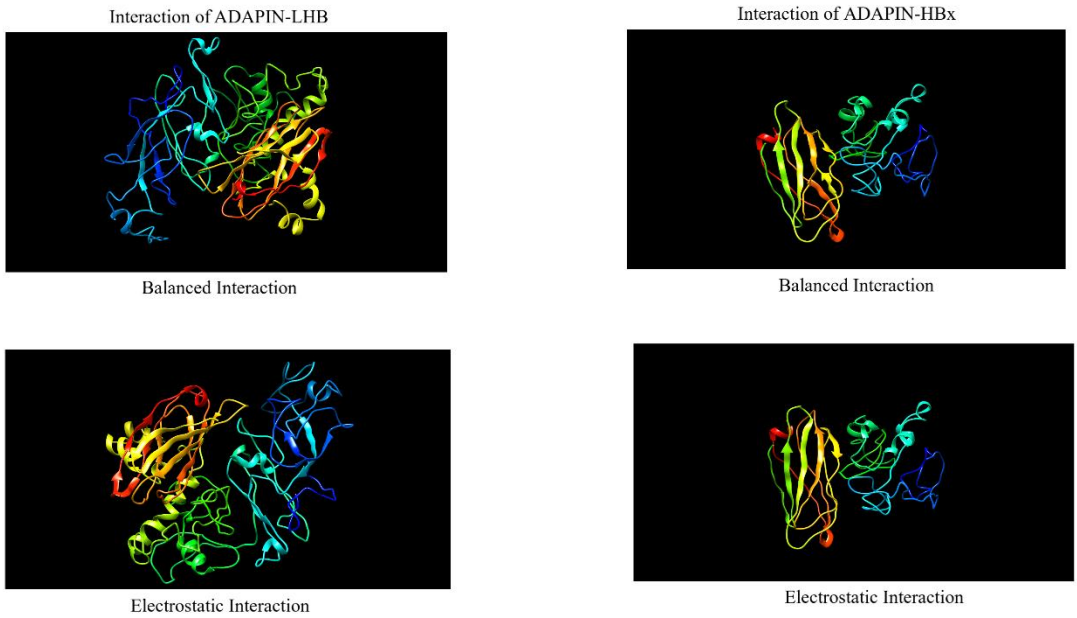

Figure 4: Protein-Protein Interaction obtained through ClusPro Software. Showing interactions of A: Gamma 2 adaptin, in blue interacting through Gamma-adaptin ear domain with the Hepatitis B virus X protein, in green and red colour B. Gamma 2 adaptin, in blue interacting through its Gamma-adaptin ear domain with the Hepatitis B virus S protein's Large subunit, in green and red colour. Protein-Protein Interaction obtained through ClusPro Software.

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Table 2: CLUSPRO DOCKING SCORES OF A: Adaptin-HBx Complex and B: Adaptin-LHB: The tables represent the cluster which is the best pose of docking; the members where the lowest possible number of members were in the group; Weighted score which represents the highest negative energy obtained for the corresponding pose and clusters.

A

CLUSPRO DOCKING SCORES ADAPTIN-HBx			
Cluster	Members	Representative	Weighted Score
0	111	Centre	-789.7
0	111	Lowest Energy	-789.7
1	83	Centre	-704.5
1	83	Lowest Energy	-756.7
2	59	Centre	-705.7
2	59	Lowest Energy	-820.8
3	56	Centre	-636.6
3	56	Lowest Energy	-823.7
4	56	Centre	-656.4
4	56	Lowest Energy	-796.1
5	42	Centre	-730
5	42	Lowest Energy	-771.7

B

CLUSPRO DOCKING SCORES ADAPTIN-LHB			
Cluster	Members	Representative	Weighted Score
0	298	Centre	-601.5
0	298	Lowest Energy	-718.9
1	229	Centre	-608.1
1	229	Lowest Energy	-667.2
2	220	Centre	-602.3
2	220	Lowest Energy	-648
3	158	Centre	-617.6
3	158	Lowest Energy	-716
4	91	Centre	-687
4	91	Lowest Energy	-687
5	3	Centre	-607.2
5	3	Lowest Energy	-607.2

2.5.2. HDOCK

Second tool under consideration was results generated through HDOCK. The HDOCK server Carries out the homology search, template-based modelling, structure prediction, macromolecular docking, biological information incorporation and job management for robust and fast protein–protein docking. HDock demonstrated that the binding energies were nearly comparable for interaction of Gamma 2 adaptin with HBX and LHB protein. The binding energy between HBX and Adaptin has the value of -270 with the RMSD value of 82.83 Angstrom whereas LHB and Adaptin gave binding scores as -222 with the RMSD value of 39.77 Angstrom. Here the binding energy for Adaptin-HBx complex is more negative than Adaptin-LHB complex however the RMSD value for the latter is less (Figure 5). The three-dimensional model of the docking complex A: Gamma 2 adaptin, in blue interacting through Gamma-adaptin ear domain with the Hepatitis B virus X protein, in green and red colour B. Gamma 2 adaptin, in blue interacting through its Gamma-adaptin ear domain with the Hepatitis B virus S protein's Large subunit, in green and red colour. The background non interacting molecules are shown in grey.

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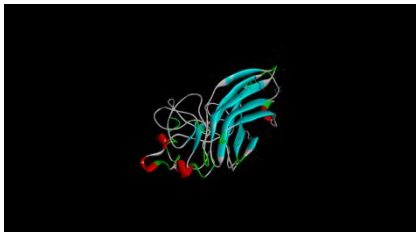
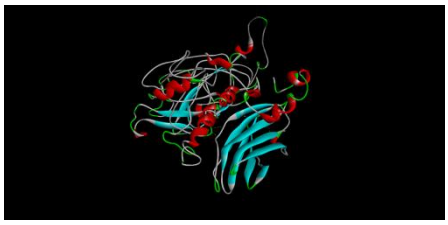
A.  B. 

Figure 5. The three-dimensional model of the docking complex A: Gamma 2 adaptin, in blue interacting through Gamma-adaptin ear domain with the Hepatitis B virus X protein, in green and red colour B. Gamma 2 adaptin, in blue interacting through its Gamma-adaptin ear domain with the Hepatitis B virus S protein's Large subunit, in green and red colour. The background non interacting molecules are shown in grey.

2.5.3. HEX

Third software that we used was Hex docking software which demonstrated results which were again acceptable from the in-silico interaction point of view. Hex can superimpose pairs of molecules based upon the knowledge of their 3D shapes only. High-resolution spherical polar docking correlations are performed over the resulting receptor surface patches, and candidate docking solutions are refined by using a novel soft molecular mechanics energy minimization procedure. The final energy score derived from Hex analysis for Adaptin-HBx complex was -558.4. Clustering found 1781 clusters from 2000 docking solutions in 0.81 seconds. Initial rotational increments (N=18) Receptor: 812 (27Mb), Ligand: 812 (27Mb) Applying 812+812 coefficient rotations on 8 CPUs for N=18. Done 1624 rotations in a total of 0.13s (12992/s). Counted 18 +ve and 13 -ve formal charged residues: Net formal charge: 5.

On the other hand, the Hex analysis for Adaptin-LHB complex was -361. Clustering found 1779 clusters from 2000 docking solutions in 1.98 seconds. Initial rotational increments (N=18) Receptor: 812 (27Mb), Ligand: 812 (27Mb) Applying 812+812 coefficient rotations on 8 CPUs for N=18. Done 1624 rotations in a total of 0.09s (17277/s). Counted 17 +ve and 13 -ve formal charged residues: Net formal charge: 4 This indicates towards HBx being a potent interacting partner for the host Adaptin molecule. In Figure 6 The three-dimensional model of the host protein Gamma 2 adaptin and the viral protein HBx, in two configurations A: This configuration represents the host protein on the left and the viral protein on right before the docking was performed using Hex. B: This configuration represents the energy difference values as well as change in distance between the host protein and the viral protein after docking using Hex.

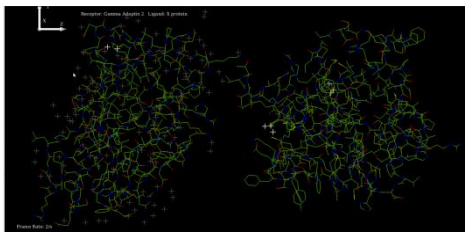
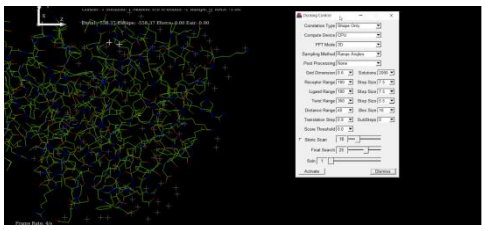
A.  **B.** 

Figure 6. The three-dimensional model of the host protein Gamma 2 adaptin and the viral protein HBx, in two configurations A: This configuration represents the host protein on the left and the viral protein on right before the docking was performed using Hex. B: This configuration represents the energy difference values as well as change in distance between the host protein and the viral protein after docking using Hex.

The overall assessment demonstrates HBx to be a probable interactor and may be considered further for in vitro validation studies with respect to its interaction with the Gamma 2 adaptin molecule during the pathogenesis.

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3. CONCLUSION

Hepatitis B virus is still being explored for its precise utilisation of the host clathrin mediated pathway. Since clathrin mediated pathways are one of the most fundamental pathways of the host which is being utilised by the virus, it becomes imperative to know the relevant host viral protein interaction pertaining to this pathway. The study emphasizes that HBx protein, with its regulatory role in establishing a successful state of the infection, may play an important role in regulating the clathrin mediated entry of the virus. Usually for association and dissociation of a complex formed between viral and host proteins, a regulatory viral protein is necessary for the propagation of pathogenesis. The viral S protein provides the primary protein to interact with the hub proteins but viral regulatory protein is also necessary to regulate these interactions. The studies have successfully established the role of S protein with adaptor proteins during the clathrin mediated endocytosis. X protein of Hepatitis B virus (HBx) is a non-structural regulatory protein. During pathogenesis, the precise role of this protein is still elusive, nonetheless there are multiple pathways where this protein has been found to be involved. Hbx has been found in the modulation of transcription, apoptosis, cell cycle progress as well as genetic stability by modulating the host factors directly or indirectly. This protein has been found to be involved in the amelioration of the host's defense pathways which is an imperative aspect for the virus to establish itself within the host system. Through this process the X protein regulates the processes facilitating the successful pathogenesis with the final eventuality of hepatocellular carcinoma and cirrhosis.

However, there is scarce data pertaining to the function of the HBx protein in the duration of the whole clathrin mediated pathway. Therefore, this study is a small endeavour to bring forth relevance of other viral proteins such as the viral regulatory protein i.e., the HBx protein and its role in the clathrin mediated endocytosis. Despite these recent discoveries, the intracellular trafficking events, which are critical for the initiation of a productive infection by providing the appropriate environment for virus uncoating and nucleocapsid release, have remained completely obscure for HBV [17].

The important aspect that the study highlights is that the same protein does not interact with Gamma-1-adaptin which closely resembles Gamma-2-adaptin thereby highlighting the specific nature of the interaction. This gives us further insight as to the specific nature of interaction. In-silico approach is used to demonstrate that the role of X protein with Gamma 2 adaptin can be one of the potential interactors of this pathway. The greatest challenge in working with hepatitis B virus is that there is a dearth of stable systems in vitro that can mimic HBV infection to foster key questions in HBV pathobiology [18]. Therefore, finding novel interactions with respect to HBV becomes quite tedious and challenging. Therefore, the in-silico approach may save a lot of time, resources and rescue the directionless search for novel aspects of HBV. Such studies can provide directional insight towards the potential areas. However, this study is just indicative and most certainly requires experimental validation.

4. MATERIALS AND METHODS

4.1. PPIN (Protein-protein Interaction Network)

A protein-protein interaction network is a network of diverse proteins connected to each other through common functionality or physical interactions. The computational analysis of PPI networks begins with the simple graphical illustration of the proteins. The two or more proteins in a network are the nodes which are interlinked to each other though the edges in a PPI network arrangement. In the due course of the examination of the network, we can generate a variety of conclusions such as the adjacent proteins may share a common functionality or a dense collection of proteins in a cluster may represent a protein complex. An insight into the topological placements of the proteins in a network can provide us with a fair estimation of the possible novel functions of the same proteins in complexes. Such topological analysis leads us towards the prediction of new interactions which may be novel and useful based upon the exclusive structural information provided by the PPI network topology. Based on this principle we have used STRING database for our topological analysis for the generation of new insights into the already PPIN network data available [19].

4.1.1. STRING database

The STRING database intents to integrate all aspects of the known as well as the predicted associations amongst the diverse proteins which includes both physical as well as functional associations. To accomplish

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this, STRING database assimilates and provides scores to the evidences acquired from various sources such as data from the scientific literature using automated text mining, computational interaction predictions from co-expression and from conserved genomic context, several databases containing data pertaining to experimental interaction and annotated complexes/pathways, and information of the interaction evidence from one organism to another. Creation of Protein-protein interaction (PPI) network using STRING version 10. The STRING resource is available online, at https://string-db.org/. The STRING version 10 database was used online to generate a PPI network using Hepatitis B virus S protein as the query protein and selecting Homo Sapiens as the species. The results showed eight direct interactors. After studying the nature of each direct interactor, we short listed AP1G2 - AP-1 complex subunit gamma-like 2 interactor (alternately known as the Gamma 2 adaptin [https://www.genecards.org/cgi-bin/carddisp.pl?gene=AP1G2]) and generated the PPIN. The interactions PPI network obtained in STRING generated ten known functional associations shown as nodes connected by edges (Figure 1). A more refined network was generated by changing the parameter setting to high confidence score, functional and physical interaction and experimental and co-occurance data (Figure 2). This gave us the most well studies experimentally three adaptor proteins AP1G2, AP1M2 and AP1S2, which seemed to be involved in clathrin-mediated endocytosis. Upon further investigation of all the evidences provided by the database, we shortlisted AP1G2 adaptor protein due to its exclusivity as an interactor with the Large subunit (LHB) of the HBV S protein during the HBV infection [20].

4.1.2. Modelling of viral proteins

The Hepatitis B Viral Proteins such as X and the Large subunit of the S proteins do not have experimentally verified crystal structures on PDB. In order to model the 3-dimensional structures, we obtained the amino acid sequence from the uniprot for the viral proteins: HBx: Q8V1H6_HBV and LHB: Q67953_HBV.

4.1.3. Protein structure prediction (2D and 3D)

As no structural information is reported in the publicly available database i.e., PDB here we have adopted to model the protein 2D and 3D structure of the viral HBx and LHB proteins respectively. For protein secondary structure we have used PORTER 4.0 [21], that uses an ab-initio based method to predict protein secondary structure and can be accessed at http://distillf.ucd.ie/porterpaleale/. In case of protein 3D structure, we have used I-TASSER (Iterative Threading ASSEmbly Refinement) uses a hierarchical approach to protein structure prediction and structure-based function annotation can be accessed at https://zhanglab.dcmb.med.umich.edu/I-TASSER/ [23, 24]. It generates 05 best models and can be assessed on the basis of RMSD, TM-Score and C-score (confidence score).

4.1.4. Protein model validation

For protein 3D structure validation, we have used the most primitive and valid analysis i.e. Ramachandran plot analysis, comparing the -phi (φ) and psi (ψ)- of the residues (amino acids) contained in a peptide. (Table 1) By making a Ramachandran plot, we can determine which torsional angles are permitted and can obtain insight into the structure of peptides. We have derived the Ramchandran pot data and listed in the table #. The Ramachandran analysis is carried out using Procheck server.

4.1.5. Protein-protein docking

The protein gamma 2 adaptin shortlisted from the STRING 10 database was subjected to insilico docking against the viral HBx protein. The three molecules HBx, LHB and Gamma 2 adaptin were prepared by removing their water molecules and adding H bonds to their structures. Docking was done to compare the binding energy values between LHB-Gamma 2 adaptin complex and HBX-Gamma 2 adaptin complex. These three proteins were then subjected to three insilico PPI docking tools:

4.1.5.1 Cluspro 2.0: ClusPro is a widely used software for direct docking of two proteins. ClusPro first performs rigid body docking by sampling billions of conformations and then clusters 1000 lowest-energy structures based on root-mean-square deviation. It also refines the selected structures by minimizing the CHARMM energy. The top ten docking structures were selected based on lowest energy, and the protein interactions calculator was used to identify different types of interactions including hydrophobic (interaction within 5 A), hydrogen bonds, cation-pi, ionic and aromatic–aromatic interactions in all

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the ten models [24]. The PDB structure of Gamma 2 adaptin was uploaded as protein and the predicted structures of the viral proteins were uploaded as ligands.

4.1.5.2 Hex 8.0: Hex 8.0 free software was downloaded from online source hex-8.0.0-win-cuda50.exe. The default settings were used for the program to perform an initial Steric Scan at N=18, followed by a Final Search at N=25, using just the steric contribution to the docking energy. In this mode, about all but the top 30,000 orientations are discarded after the Steric Scan. The Steric Scan (N=18) phase of the docking calculation will be performed at (1+40)/0.75=53 intermolecular separations, in +/- steps of 0.75 Å starting from the current distance posted in the R slider in the bottom border of the main window. The Final Search (N=25) phase will be applied to the the highest scoring scan orientations in steps of 0.75/2 Å, as described above. The rotational search will use angular increments of about 7.5 degrees in each of the two ligand and receptor rotational angles, and in steps of 5.5 degrees about the twist angle. Most conventional FFT docking algorithms have to use rather large grids (e.g. 0.6Ångstrom cubes) because the grid must accommodate all possible translations of the ligand about a stationary receptor. Here, the grid only needs to contain the larger of the two molecules so that much finer sampling grids are feasible.

4.1.5.3 Hdock: The HDOCK server is to predict the binding complexes between two molecules like proteins and nucleic acids by using a hybrid docking strategy. The HDOCK performs global docking to predict the binding complexes between two molecules. Therefore, no information about the binding site is necessary for the docking job. However, the server also gives users the option to specify the binding site residues if such information is available, such that the predicted models will have a higher accuracy [14]. The Gamma 2 adaptin PDB ID was uploaded as the receptor and the modelled viral proteins were uploaded as the ligands. The rest of the parameters were kept on the default settings.

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