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ORIGINAL ARTICLE

Structural Evaluation of Interaction between HCV Enveloped Protein E1 and P2X4 Receptor on Hepatocytes

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Abstract:

Background: Since the hepatitis C virus (HCV) is the primary cause of liver-related illnesses like chronic hepatitis, cirrhosis, fibrosis, and hepatocellular carcinoma, it poses a serious threat to global public health. HCV structural envelop protein E1 is the primary viral entrance protein. P2X receptors are ligand-gated by extracellular ATP and are non-selective ion channels. Among all P2X receptors, P2X4 and P2X7 are the proteins that are most frequently expressed in the liver.

Methods: This work used immunostaining to describe the P2X4 receptor in the Huh7 cell line. By measuring Ca2+ in the presence of an agonist using the Flex Station III, the functional activity of P2X receptors was validated (ATP). Using a zebra fish model as a starting point, molecular operating environment (MOE) software was used to construct the human P2X4 receptor (through homology modeling). On the website High Ambiguity Driven Biomolecular Docking (HADDOCK), P2X4 and E1 protein-protein interactions were investigated (PPIs).

Results: Our results demonstrate many sites of interaction between P2X4R and the HCV envelop E1 protein. In the Huh7 cell line, the P2X4 receptor was discovered and functionally activated. Additionally, interactions between the human P2X4 receptor and the HCV E1 protein may be indicative of viral entry and may one day be used as a therapeutic target.

Keywords: Hepatocellular cancer, the Hepatitis C virus, Docking, and Adenosine triphosphate.

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Introduction

The Hepatitis C virus (HCV) is a serious public health problem across the world because it causes chronic hepatitis, cirrhosis, and hepatocellular carcinoma (1). HCV is a member of the Flaviviridae family of RNA viruses (has a 9.5 kb genome and a positive single-stranded RNA that encodes a large polyprotein that is cleaved to create six non-structural proteins (NS2, NS3, NS4A, NS4B, NS5A, NS5B) and four structural proteins (Core, E1, E2, and P7). These viral proteins are in control of a number of different cellular processes in addition to viral replication (2).

The most crucial HCV structural proteins for viral entrance are the envelope proteins. A transmembrane glycoprotein with a C-terminal domain that controls membrane permeability and interaction is the envelope protein 1 (E1) of the HCV (3).

It is believed that E1, a fusigenic region of the HCV envelope composed of 4-5 N-linked glycans, mediates interactions between the virion and several cell surface receptors (4). Consequently, it is essential to target virus envelope proteins in order to limit viral invasion (5). One of the best ways to create new treatment approaches is to comprehend the viral entrance mechanism. On the other hand, viral fusion is a complicated process that involves fusion glycoproteins that are present on the viral envelope.

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Non-selective ion channels known as P2X receptors respond to extracellular ATP as a ligand. Each P2X channel is created by the hetero-oligomeric assembly of three P2X protein subunits (6).

When Kawate T et al. (2009) released the solved crystal structure of the zebrafish P2R4 ion channel in closed state, they validated this trimeric stoichiometry (7). The P2X receptors with the greatest expression in the liver are P2X4 and P2X7. All P2X receptor subtypes are expressed by parenchymal cells or hepatocytes, with the exception of P2X5 and P2X6 (8). Smooth muscle cells, cholangiocytes, liver fibroblasts, endothelial cells, immune cells, and immune cells are non-parenchymal cells that express several P2X receptors. It is possible that P2X receptors regulate various aspects of liver physiology given that they are present in a range of functionally specialized hepatic cells.

In order to have a range of effects, ATP binds to and activates P2X receptors. Prior research has connected P2X receptors to the regulation of apoptosis, fibrosis, inflammation, and cancer. In a recent study, it was discovered that the Huh-7/E1E2 cell line, which is capable of stably expressing the HCV structural proteins E1E2, increased the expression of the P2X4 receptor gene by 6.2-fold. By engaging with a number of host cell receptors that stress the hepatocytes and increase ATP release, the HCV/E1 protein-protein interaction (PPI) studies with the P2X4 receptor seek to better understand viral entrance into the host cell. This study will contribute to the confirmation of the hypothesis that purinergic receptor expression on hepatocytes is altered by HCV envelope protein E1.

Materials and Methods

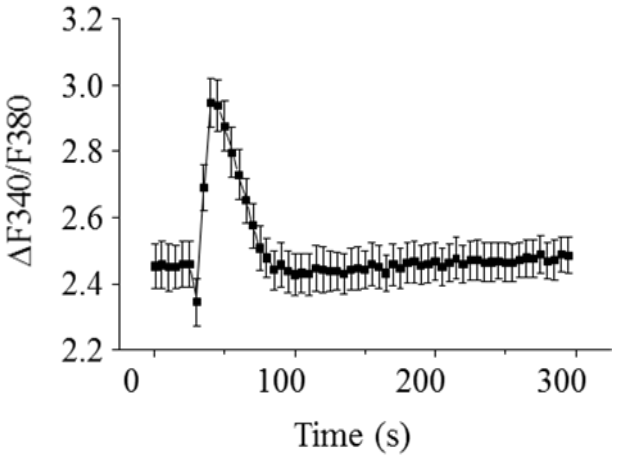
Immunocytochemistry:

Huh7 cells were used to characterize the presence of P2X4 receptor on liver cells. Cells were incubated overnight on a coverslip in cell culture medium in 24-well plates. After 24hrs, cell was fixed with the help of 4% paraformaldehyde for 10 min. Cells were permeabilized for 10 minutes with 1% Triton X-100 in PBS (PBS-T) and blocked for 30 minutes with 1% BSA in PBS. Primary antibody from rabbit anti-P2X4 (Almone) was used at a dilution of 1: 100 and incubated overnight at 4°C. The cells were washed with PBS-T the next morning before being incubated for 1 hour at room temperature with a 1:1000 dilution of FITC-conjugated anti-rabbit IgG secondary antibody (Sigma). The cells were stained with DAPI (4',6-diamidino-2-phenylindole) after washing and drying (DAPI). EVOS Cell Imaging System fluorescence pictures were combined using Image J software (Thermo Fisher Scientific).

Measurement of Ca2+

The ATP-induced activity of the P2X4 receptor was measured using the Flex station III by monitoring the change in Ca2+ level. Overnight, cells were incubated at a rate of 40,000 cells per well. The cells were rinsed the next morning with a standard buffer solution containing Ca2+. In the incubator, the cell was treated with Fura -AM-containing buffer for one hour. The intensity of the fluorescence was measured at 340 and 380nm, with an emission wavelength of 510nm. The ATP-induced Ca2+ change was denoted by a change in F340/F380 shown in Figure 1.

Figure:1 ATP induced Ca2+ response to express the functional activity of P2X4 receptors.



Cell proliferation assay:

To determine the effects of agonist on cell proliferation, the cells were seeded on 24-well plates at a density of 1 104 cells per well and incubated in culture conditions without or with ATP supplementation for 0, 24, 48, and 72 hours. SYBER Green was used to stain the cells, and an IncuCyte ZOOM® live-cell imaging device was used to collect the cells in four locations (Essen BioSceicne). The number of cells in the presence of the agonist was determined as a percentage of the number of cells under control conditions using the Image J software to count the cells.

Modelling of human P2X4 using zebrafish P2X4 model as template:

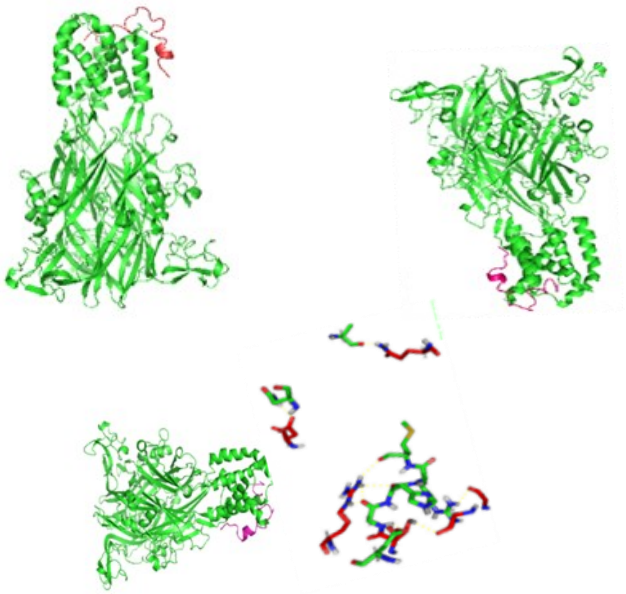
The structure of crystals Uniport (www.uniprot.org) was used to retrieve the human P2X4 sequence (ID No. Q99571). Software called the molecular operating environment (MOE) was used to align the sequences (version 2013 from the Chemical Computing Group). The sequence of zebrafish P2X4R is most comparable to that of human P2X4R, and its three-dimensional structure is unique (ID: 3I5D) was utilized to simulate human P2X4R homology (9). P2X4R homology modelling was performed using MODELLER (10). Based on the RMSD between corresponding residues in the models, fifty models were developed and categorized into groups. Based on the potential energies and ERRAT, MODELLER, and MOE scores, representative models were selected from each cluster.

Loop Refinement:

The ERRAT score for the P2X4R projected structure's human loop refinement was improved using MODELLER (11). Based on their root mean square distance, models were categorized (RMSD). For the purpose of selecting a representative model, clustered models were considered. The ERRAT score and MODELLER energy ratings were utilized as model assessment criteria.

Protein-Protein interaction

Utilizing High Ambiguity Driven Biomolecular Docking



(HADDOCK), P2X4 and E1 PPIs were explored (12). The CPORT (Consensus Prediction of Interface Residues in Transient Complexes) algorithm was used to forecast likely interface residues in each structure (13). CPORT is a method for predicting protein-protein interface residues. It is a consensus predictor that employs six ways to interface prediction. The HAD- DOCK Programme has been discovered to use CPORT to produce accurate estimates for residues.

Results

Comparative modeling of P2X4 protein

Comparative modeling of the zebrafish P2X4 protein was used to assess the interaction with HCV envelop protein E1. To construct homology models, the crystal structure of P2X4 zebrafish (ID: 3I5D) was utilized as a template. MODELLER was used to create fifty models. Ten models were chosen for further investigation based on the ratings reported by ERRAT, QMEAN, and MODELLER. ERRAT scores ranged from 70 to 80. Based on the highest ERRATs score and the lowest energy values represented in the best model was selected.

Loop refinement of human P2X4R to increase the quality factor:

MODELLER was used to construct 20 P2X4 loop models. MODELLER produced a graph that contrasted discrete optimized protein energies (DOPE) with molecular PDF energies (molpdf). 10 clusters of models were created using RMSD clustering, with the top ten models in each cluster having the best RMSD values and MODELLER reported energy ratings.

Protein–Protein interaction studies of HCV Envelop protein E1 and P2X4

The structures were grouped by HADDOCK into 1 cluster, which accounted for all of the water-refined models produced by HADDOCK. The interface is automatically generated based on all observed interactions, and the clusters are calculated using HADDOCK's interface-ligand RMSDs. Using the most effective HADDOCK model, the various structural analyses (FCC, i-RMSD, and l-RMSD) are carried out (the one with the lowest HADDOCK score). In order to ascertain the probable interactions in the docked protein, the structure was studied in Pymol.

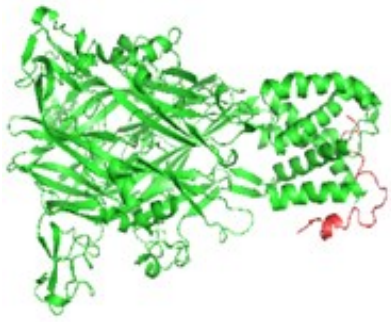


Figure:2 Structural Proteins P2X4 and E1.

Viral E1 can bind to liver cell surface receptors via 3.9 Ao polar interactions between amino acid residues, according to PPI experiments on P2X4 and E1show in figure 2. The C-terminus of the P2X4 receptor protein had the amino acids Gly-960, Gly-641, ASP-974, and ASP-648, while the N-terminus of viral E1 contained the amino acids ALA-17, ASN-12, MET-5, ARG-4, and HIS -3. Crystallization of the viral E1 protein and the docked liver receptor P2X4. Above are depicted the largest structures as well as potential relationships between various polar zones. Figure 3 shows interactions between P2X4 and E1 at 3.90 Angstroms in a schematic representation of the complex. A huge protein called P2X4 with approximately 970 residues is shown in red, whereas a little protein called E1 with only 26 residues is shown in green. The interaction's active residues are highlighted.

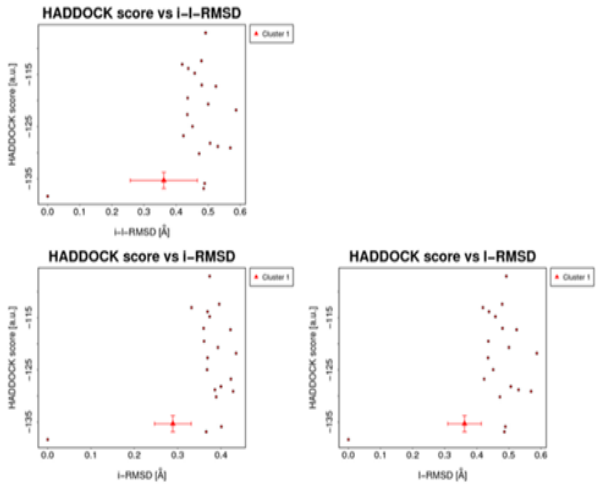
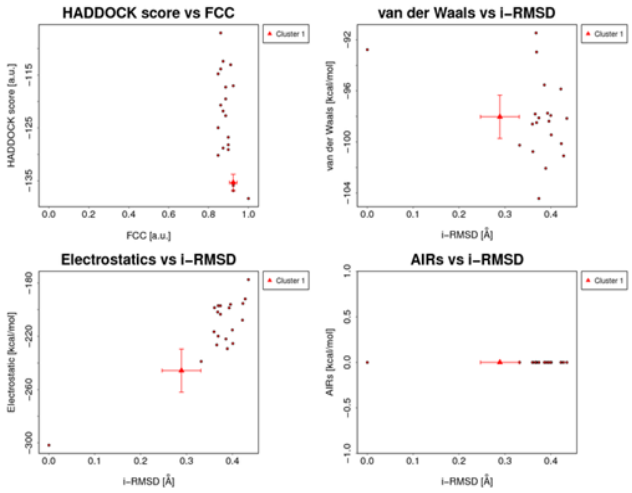


Figure:3 The following conclusions and illustrations are based on HADDOCK's water-refined models. The interface is generated automatically based on all observed interactions to create the clusters, which are displayed in colour in the graphs. Using the most effective HADDOCK model, the various structural analyses (FCC, i-RMSD, and l-RMSD) are carried out (the one with the lowest HADDOCK score)

Discussion

In order for enveloped viruses to infect eukaryotic cells, the viral and plasma or endosomal membranes must fuse (REF). This process is catalyzed by highly specialized viral envelope glycoproteins, which overcome the reaction's intrinsic energy restrictions (REF). Additional parts of these glycoproteins are needed to drive and finish the process, even if the fusion peptide is largely necessary for the first stages of membrane fusion (REF). There may be a way to lower the energy barrier needed for fusion by preventing pretransmembrane (PTM) regions from being primary drivers of phospholipid bilayer instability (14, 15). The region of the E1 glycoprotein near the TM domain, between residues 309 and 340, is thought to play a role in membrane instability as well as pore development and enlargement in all HCV strains, similarly to the pretransmembrane and/or loop domains of class I fusion proteins (16). It has been discovered that CD81, a host membrane-spanning protein that was once suspected to being an HCV receptor, is required but not sufficient for infection (17). Other potential receptors involved in the productive infection process include the recently discovered Claudin 1 and Occluding factor as well as the class B, type I scavenger receptor SR-BI and the low density lipoprotein receptor LDL-R (18, 19) has been demonstrated in a number of scenarios, however the exact topology of the event is yet unknown. Numerous P2X receptor isoforms are expressed throughout the body (20). Rat liver cells and rat hepatocytes contain the unique P2X receptor genes P2X1, P2X2, P2X3, P2X4, and P2X7, according to previous studies (21).



In a recent work, we found distinct P2X receptor transcripts in Huh-7 and Huh-7/E1E2 cells (22). In order to build the groundwork for viral exploitation of these receptors to increase disease severity, the current experiment seeks to investigate any potential associations between P2X4 receptors on the human liver surface and HCV E1 protein. The study of protein-protein interaction studies can be performed with repeated observations of critical assessment of anticipated interactions thanks to the accessible crystal structure of the HCV E1 viral entry protein and the development of a suitable P2X4 model from an existing Zebrafish model (CAPRI). ERRAT, QMEAN, and MODELLER outputs were used to choose ten models for additional examination. The ERRAT ratings ranged from 50 to 60. The model chosen as the best had the highest ERRATs score and the lowest energy values.

PPI was examined using the standard Guru interface HAADOCK, but the findings were odd, showing implausible interactions between human P2X4 receptors and HCV E1. Later research shown that loop refinement could interfere with interacting residues by reducing stretch and tension. To our surprise, when the PPIs were performed without refinement, the Guru interface was used to validate the real links between two proteins, and the HADDOCK refinement interface was used to validate the protein interactions and structure. Viral E1 can bind to liver cell surface receptors via 3.9 Ao polar interactions between amino acid residues, according to PPI experiments on P2X4 and E1. The C-terminus of the P2X4 receptor protein had the amino acids Gly-960, Gly-641, ASP-974, and ASP-648, while the N-terminus of viral E1 contained the amino acids ALA-17, ASN-12, MET-5, ARG-4, and HIS-3.

Conclusions

The results of this study suggest that the HCV virus exacerbates disease pathology by using open receptors on the cell surface. These receptors can be exploited to target the escalating circumstances in liver disease even if their potential for viral entry into liver cells has not been investigated.

Availability of data and materials:

On demand data is available.

Conflict of interests

The authors affirm that they do not have any conflicting interests.

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Author's contributions

MK and MSN were involved in the execution of the project. AZ designed, executed the study and wrote the manuscript. UH and IH helped in organization of data. SK and SAZ helped in the editing. All named authors have read and approved the final version of the manuscript.

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