Virtual Docking of VBP1 with HBX and NFκB protein to study activation of NFκB in the regulatory mechanism of Liver Cancer

Abstract

Hepatitis B virus (HBV) X protein (HBx) is essential for virus infection and has been implicated in the development of liver cancer associated with chronic infection. It can interact with a number of cellular proteins. In cell culture, it exhibits pleiotropic activities, among which it interferes with cell viability and stimulates HBV replication. The von Hippel-Lindau binding protein 1(VBP1) has a key role in HBx-mediated nuclear factor kappa B (NFkB) activation. VBP1 plays as a coactivator of HBx in the activation of NFκB binding. Because of priority of site of docking HBx with NFkB and between NFkB and VBP1, specific sites were selected for docking. *In silco* results suggested thatVBP1 has a key role in HBx-mediated NFκB activation.

Key Words : Hepatitis B virus (HBV) X protein (HBx) ; Nuclear Factor kappa B Activity (NFkB); von Hippel-Lindau binding protein 1(VBP1); Docking; Liver Cancer

Introduction

Millions of people were affected by chronic infection of hepatitis B virus (HBV) which causes liver diseases, including cirrhosis and hepatocellular carcinoma (Parkin *et al*., 1999). The hepatitis B virus (HBV) X protein (HBx) has stimulated HBV for transcription and replication in hepatocytes *in vivo*. The function of HBx may be crucial to its stimulatory effect on HBV transcription and replication (Gong *et al*., 2013). HBx has been reported to be capable of activating several signal transduction pathways, such as mitogen-activated protein kinase, Ras-Raf-mitogen-activated protein kinase, and JAK/STAT signaling pathways to affect several cellular processes, including proliferation and differentiation [Bandar *et al*. 2004,Bontron *et al.* 2002].HBx also participates in inducing cell death by the death receptor pathway or affecting mitochondrial pathophysiological microenvironment to mediate apoptosis [Bressac *et al.* 1990, Chen *et al.* 1993, Colgrove *et al.* 1989, Dandri *et al.* 1998]. Moreover, HBx plays an important role in tumor spreading by enhancing cellular migration through upregulation of MMP-9, MMP-3, MT1-MMP and COX-2 [Doitsh *et al* 1999, Doria *et al* 1995, Duflot *et al* 1995, Ganem *et al*

2001]. VBP1 is reported to be localized in the cytoplasm, especially in the perinuclear region and skeletal muscle, heart, brain, kidney, spleen, lung, and liver (Brinke *et al.* 1997). HBx is a promiscuous protein containing various functions by interacting with a multitude of cellular proteins. Since VBP1 is a bona fide cellular protein interacting with HBx, the reciprocal effects of each protein in the regulation of other protein needs to be tested.The docking interaction between different models of HBx & VBP1 and between VBP1& different types of NFkB have been identified in the present study. It is important because the NFκB has been shown to be associated with tumorigenesis by inflammation, anti-apoptosis, and cell proliferation. The constitutive activation of NFκB has been reported in several human cancers.

Materials & Methods

Protein sequence of HBx has been retrieved from NCBI (The National Center for Biotechnology Information, www.ncbi.nlm.nih.gov/). Geno3d was used for modeling (http://geno3d-pbil.ibcp.fr/); four sequences are modeled ABR68892.1, Q4R1S1.1, P0C685.1, Q99HR6.1. Three dimensional X ray crystallized structure of Von Hippel-Lindau protein (VBP1, PDB: 4AJY) was downloaded from the protein data bank (Van Molle, *et al.* 2012). The protein was taken as receptor protein and most suitable site was predicted by using q site finder ligand binding site prediction (http://www.modelling.leeds.ac.uk). The crystal structure of nuclear factor-kappaB ligand NFkB1 (PDB: 3QBQ, Ta *et al*., 2010), NFkB2 (PDB: 3ME4 Liu *et al*., 2010), NFkB3 (PDB: 4GIQ Nelson *et al*., 2012), NFkB4 (PDB: 3ME2, Liu *et al*., 2010) were downloaded from the protein data bank.

Because of priority of site of docking HBx & NFkB and between NFkB & VBP1 have been selected for docking. Docking study was done with Hex Server (http://hexserver.loria.fr/) for HBx & VBP1 and between VBP1& different types of NFkB. It is automated on line software with fast processing. The binding site cavity detection was performed by q site finder ligand site prediction tool. It shows E total, E shape, E force, root-mean-square deviation (RMSD), number of H-bond and interaction between interacting residues of receptor HBx, NFkB andVBP1, which indicates towards the formation of stable complex among ligand and receptor molecule. MVD visualizer is used for interaction site prediction (Thomsen and Christensen, 2006).

Results & Discussion

The four sequences of HBx (ABR68892.1, Q4R1S1.1, P0C685.1 and Q99HR6.1) are modeled by Geno3d. The modeling of proteins refers to constructing anatomic-resolution model of the target protein from its amino acid sequence. Then these models are docked to predict the intraction with HBx & VBP1 and between VBP1& different types of NFkB. The comparative results obtained from (using hex-docking server) docking simulation with different models of HBx & VBP1 and between VBP1& different types of NFkB are given inTable-1&2 respectively.

Table -1 Comparative Docking Simulation Results of selected Model of HBx with VBPI using hex-docking server.

S. No	ligands	E Total	E Shape	E Force	RMSD
1.	Model-1	-855.10	-855.10	00	-1.00
2.	Model-3	-738.20	-738.20	00	-1.00
3.	Model-4	-789.31	-789.31	00	-1.00
4.	Model-5	-472.83	-472.83	00	-1.00

Table -2 Comparative Docking Simulation Results of NFkB1, NFkB2, NFkB3 & NFkB4 with VBPI hex-docking server.

S. No	ligands	E Total	E Shape	E Force	RMSD
1.	NFkB1	-870.15	-870.15	00	-1.00
2.	NFkB2	-768.11	-768.11	00	-1.00
3.	NFkB3	-883.70	-883.70	00	-1.00
4.	NFkB4	-758.74	-758.74	00	-1.00

The interactions have been analysed to find out the residues that are involved in binding site residues and number of hydrogen bonds are involved in interaction among them.

The energy bound conformation with lower value shows hydrogen bond interactions are given in (Table -3, Figures 1). Docking energy for HBx was found favorable for VBP1 which shows that these compounds can get stuck due to positive interaction (Saxena 2013) .

Table -3 VBPI residues interact with selected Model of HBx using hex-docking server.

(highlighted residues are involved in H-bonding interaction with ligands

S. No	ligands	Interacting residues of receptor VBPI	No. of H-bond interaction
1.	Model-1	**Arg 161**, Glu 92 and Arg 82	3
2.	Model-2	**Arg 161, Gln 11, Ala 14**, Ile13, Glu 92 and Arg 82	5
3.	Model-3	**Arg 161**, Glu 92 and Arg 82	3
4.	Model-4	**Arg 161**, Glu 92 and Arg 82	3

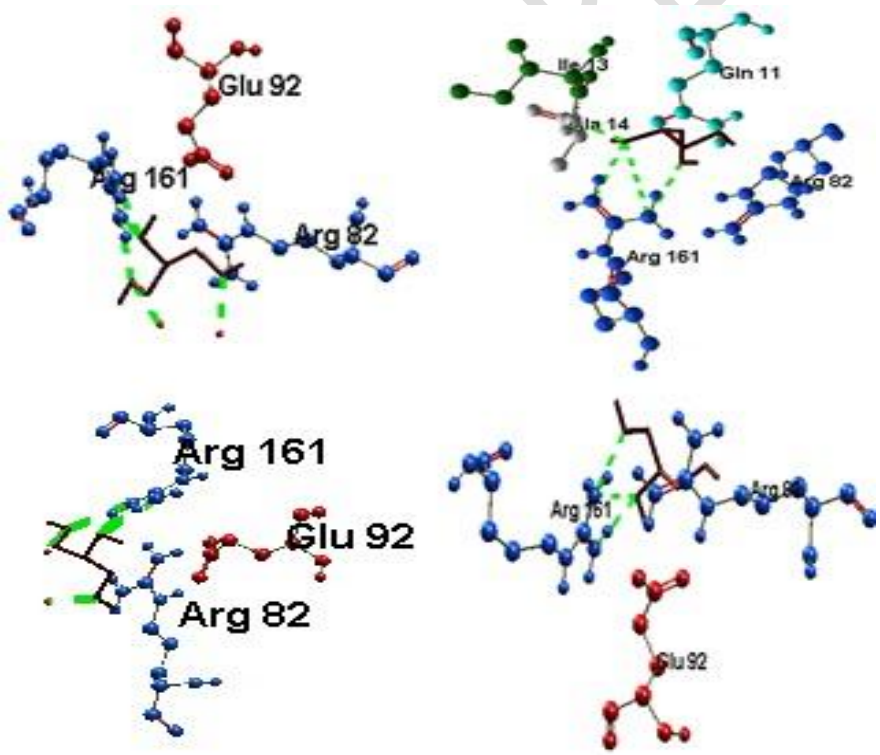

Fig- 1 Docked conformation of hydrogen bonding view of VBPI residues interact with selected Model of HBx protein at the active site cavity

The model one, three and four shows very high binding energy to bind with VBP1 and they interact with Arg 161, Glu 92 and Arg 82 residues of VBP1. All these residues involved in binding belong to the cavity-1. It forms 3 hydrogen bonds with Arg 161 (Table -3 and Fig. 1a).

While, the model two also shows very high binding energy to bind with VBP1 and it interacts with Arg 161, Gln 11, Ala 14, Ile13, Glu 92 and Arg 82 residues of VBP1. All these residues involved in binding belong to the cavity-1. It forms 5 hydrogen bonds with Arg 161, Gln 11, Ala 14 (Table -3 and Fig. 1 b). The hydrogen bonding is very significant for interaction of biomolecules.

The VBP1shows very high binding energy to bind with NFkB1 and it interacts with Arg 161,Glu 38, Glu 92 and Arg 82 residues of VBPI. All these residues involved in binding belong to the cavity-1. It forms 4 hydrogen bonds with Arg 161, Glu 38 (Table 4 and Fig. 2 a).

Table -4 VBPI residues interact with NFkB1, NFkB2, NFkB3 & NFkB4 ligands, using hex-docking server. (Highlighted residues are involved in H-bonding interaction with ligands

S. No	ligands	Interacting residues of receptor VBPI	No. of H-bond interaction
1.	NFkB1	**Arg 161, Glu 38**, Glu 92 and Arg 82	4
2.	NFkB2	**Arg 161,** Glu 92 and Arg 82, Lys 91	3
3.	NFkB3	**Arg 161,** Glu 92 and Arg 82	3
4.	NFkB4	**Arg 161,** Glu 92 and Arg 82	3

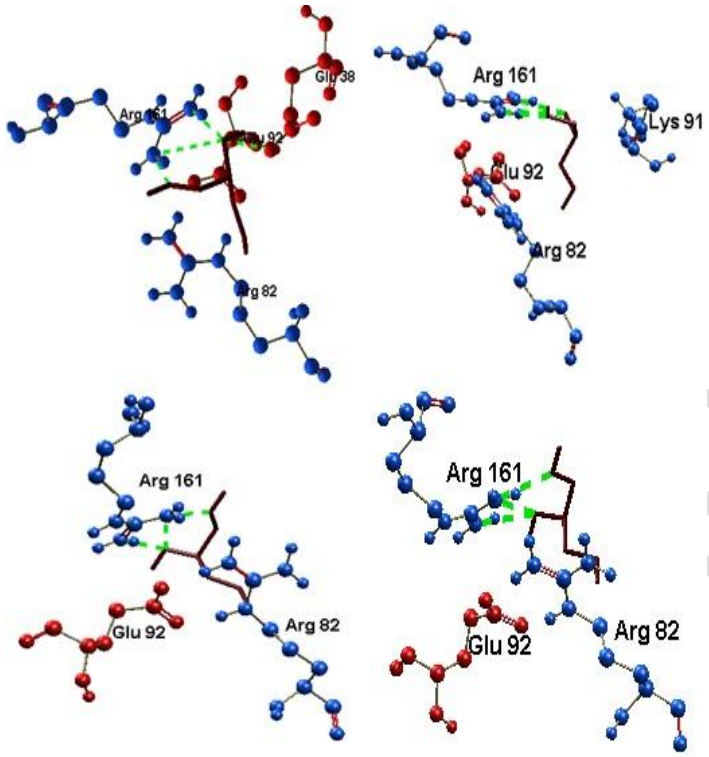

Fig – 2 Docked conformation of hydrogen bonding view of VBPI residues interact with Selected NFkB protein at the active site cavity

The VBP1shows very high binding energy to bind with NFkB2 and it interacts with Arg 161, Lys 91, Glu 92 and Arg 82 residues of VBPI. All these residues involved in binding belong to the cavity-1. It forms 3 hydrogen bonds with Arg 161 (Table -4 and Fig. 2 b). The VBP1shows very high binding energy to bind with NFkB3 & NFkB4 and it interacts with Arg 161, Glu 92 and Arg 82 residues of VBPI Fig. 2 c & Fig. 2 d respectively. All these residues involved in binding belong to the cavity-1. They form 3 hydrogen bonds with Arg 161 (Table -4). The hydrogen bonding is very significant for interaction of biomolecules.

Protein (VBP1) is interacting with von Hippel-Lindau protein (VHL) was binds with HBx (Tsuchiya *et al.* 1996). Recently it has been reported that VHL is associated with the regulation of NFκB (An and Rettig 2005).

Conclusion

VHL binding protein (VBP1) binds to HBx and VBP1binds with NFkB. In silco results indicated that VBP1 might be involved in the regulatory mechanism of HBx in the activation of NFκB. Virtual Docking of VBP1 with HBX and NFκB protein helps us to study activation of NFκB in

the regulatory mechanism of Liver Cancer. Amino Acids interactions play an important role in manifestation of Liver cancer. This analysis revealed amino acids involved in interaction among HBx, VBP1 and NFκB protein which has significance normal and abnormal regulation. VBP1 and NFκB docking revealed NFκB **interact with Arg 161, Glu 38**, Glu 92 and Arg 82 amino acids of VBP1 protein with four(4) Hydrogen bonds.

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