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Pathway Engineering, Epitope Mapping and Docking of Hepatitis B-HBx Protein.

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ABSTRACT

Hepatitis B is a severe a viral inflammation of liver. HBx protein target host proteins, involved in a variety of functions regulating transcription, cellular signaling cascades, proliferation, differentiation, and apoptosis. In the present study metabolic pathway of this protein was engineered based on available database and computational programs. The antibody binding site of HBx protein was also predicted. The predicted epitope region may facilitate effective vaccine design as well as drug discovery.

Keywords: Pathway engineering, hepatitis B, HBx, epitope, docking

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INTRODUCTION

Hepatitis B is an infectious inflammatory illness of the liver caused by the hepatitis B virus. This is a small ~3.4-kb DNA virus containing four partially overlapping open reading frames, encoding the C, S, and X proteins and a viral DNA polymerase. HBx is a multifunctional protein, which regulate cell division and cell death through unclear mechanisms [1]. The life cycle of hepatitis B virus is very complex. The virus gains entry into the cell by binding to a receptor on the surface of the cell and enters it by endocytosis. The virus membrane then fuses with the host cell's membrane releasing the DNA and core proteins into the cytoplasm. After entry into hepatocytes, HBV DNA is transported to the nucleus and converted into a covalently closed circular molecule cccDNA. The cccDNA is the template for transcription of all viral RNAs including the pregenomic RNA (pgRNA), encoding for 7 viral proteins: large, middle, and small envelope proteins (LHBs, MHBs, and SHBs) that form the surface antigen (HBsAg), the core antigen (HBcAg), the e antigen (HBeAg), the HBV polymerase, and the regulatory protein X (HBx). HBV infection leads to a wide spectrum of liver diseases raging from chronic hepatitis, cirrhosis to hepatocellular carcinoma.

A major proportion of patients with chronic hepatitis B are infected with a variant form of HBV which decreases or abolishes the production of hepatitis B e-antigen (HBeAg). Though considerable effort has been made to find suitable fragments (epitope) that can be included in vaccines to offer protection against serious life-threatening diseases, it is believed to be inadequate**.** The role of HBx as a protease inhibitor is still controversial since the existence of true serpin-like domains in the protein has been disputed [2]. It is assumed that HBV proteins target host proteins, involved in a variety of functions, thus regulating transcription, cellular signaling cascades, proliferation, differentiation, and apoptosis. More specifically the mechanism of action of HBx is still not fully understood. The objectives of the present study are to review and trace the metabolic pathway of the HBx protein and epitope designing for possible support in vaccine development.

MATERIALS AND METHODS

Navigation of KEGG Pathway database

The KEGG database [3] was surveyed for disease and pathway information. The proteins of Hepatitis B were identified and the role of HBx protein was referred that affect the immune system of the host. The binding of bacterial and human proteins is linked to apoptosis. The sequence of HBx protein was retrieved from NCBI. The metabolic steps were studied from various literature and the constituent intermediates were identified.

Construction of metabolic pathway

The metabolic pathway was constructed using protein lounge web server [http://www.proteinlounge.com]. The receptors, intermediates and inhibitory proteins were confirmed from literature sources.

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Epitope Mapping and docking

The antibody binding site of HBx protein was predicted using IEDB analysis tool [4]. The Physiochemical properties were characterized using different methods. The structure was generated using 'Ellipro' (Epitope prediction based upon structural protusion) within IEDB which will further facilitate vaccine development [5]. The best epitope region was selected by different available methods such as Chou & Fasman beta-turn prediction, Emini surface accessibility prediction, Karplus & Schulz flexibility prediction, Kolaskar & Tongaonkar antigenicity, Parker hydrophilicity prediction and Bepipred linear epitope prediction. Parameters such as hydrophilicity, flexibility, accessibility, turns, exposed surface, polarity and antigenic propensity of polypeptides chains have been correlated with the location of epitopes with different threshold values.

The 3D structures of HBx and VDAC3 proteins were obtained from PDB and docked using hex software.

RESULTS AND DISCUSSION

Construction of metabolic pathway

The constructed metabolic pathway showed step wise activity of HBx protein alongwith various interemediates leading to apoptosis. The resulting path way is given in the Fig. 1.

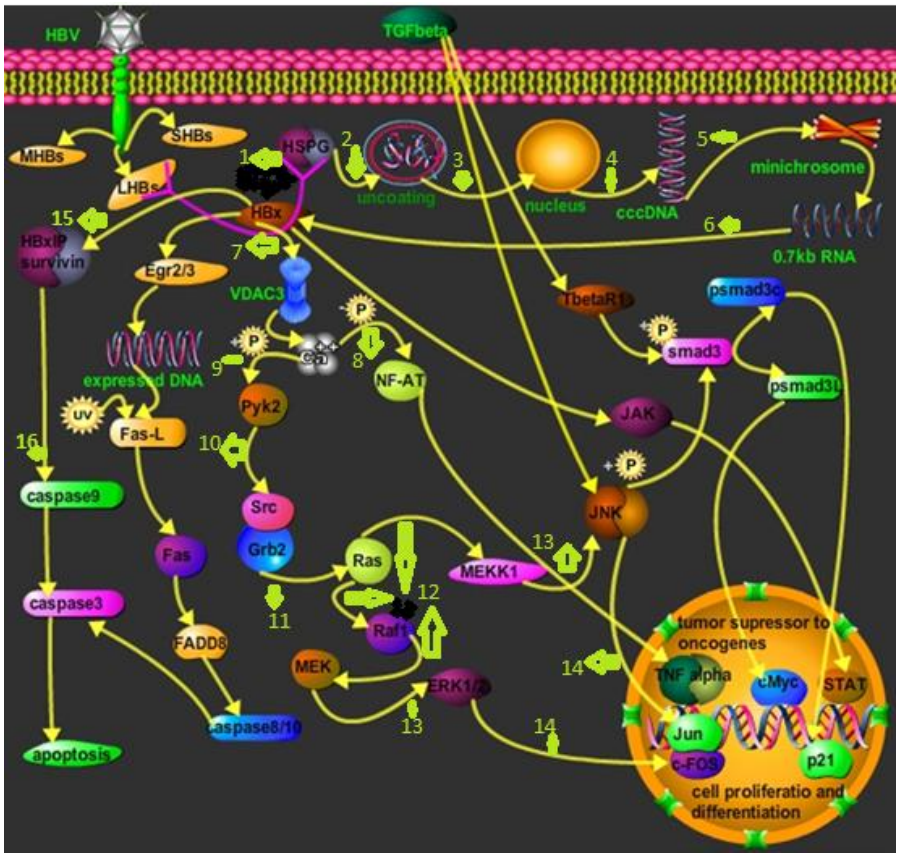

Fig. 1: Engineered pathway of HBx protein

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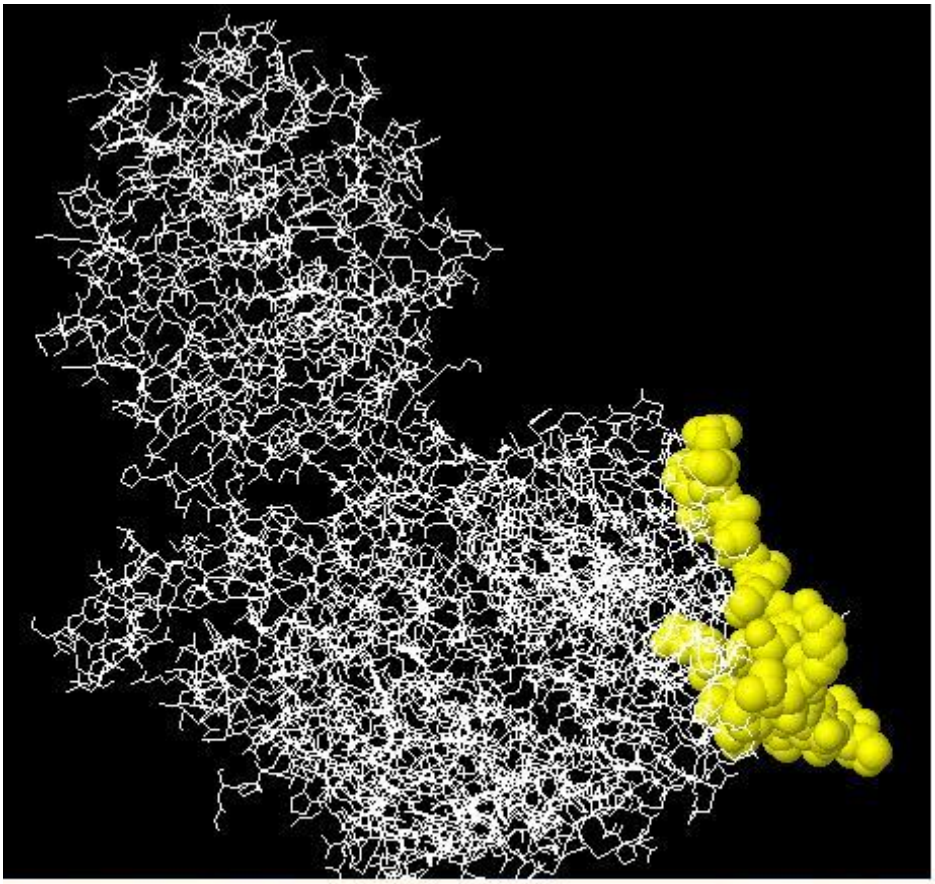
Epitope Mapping and Docking

The antibody binding site of HBx protein was predicted with its different physiochemical properties. The best considered region for epitope prediction was located. The 3D structure of the predicted epitope was obtained and visualized using JMol (Fig. 2).



Start Position	End Position	Peptide	Number of Residues
761	790	TQALSSSVSSSKLFSSSTAPHETSFGEEVE	30

Fig. 2: 3D structure of epitope region HBx

HBx regulates numerous cellular signal transduction pathways and transcription factors as well as cell cycle progression and apoptosis. Various researchers have depicted different pathway maps pertinent to HBx protein which binds with the human protein VDAC3. HBx protein modulates PI3K/Akt pathway to overcome genotoxic stress-induced destabilization of cyclin D1 and arrest of cell cycle [6], HBx induces HepG-2 cells autophagy through PI3K/Akt-mTOR pathway [7], involvement of the NF-κB pathway in multidrug resistance induced by HBx in a hepatoma cell line [8] are to mention a few. The VDAC3 is a channel protein and carries the activity of the HBx protein. The other proteins are also affected by this protein forming the major apoptotic pathways such as Ras-Raf-MAPK and Jak-STAT etc. which are responsible for the cell proliferation, inflammation, and cell death etc. Docking of these two proteins showed relative orientations of binding, molecular interaction map and possibility of suitable designing of a novel inhibitor for HBx protein.

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CONCLUSION

Numerous metabolic pathways are designed for HBx protein of Hepatitis B virus. In the current study, the pathway is redesigned referring the intermediate metabolites. Epitope mapping followed by docking was carried out to know the antigen-antibody binding site. It is expected that similar studies would help in better understanding of the pathway leading to efficient vaccine development and characterize possible biological activity of the vaccines of interest.

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