

## Review Article

# HBV Induced HCC: Major Risk Factors from Genetic to Molecular Level

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Hepatocellular carcinoma (HCC) is a deadly and emerging disease leading to death in Asian countries. High hepatitis B virus (HBV) load and chronic hepatitis B (CHB) infection increase the risk of developing HCC. HBV is a DNA virus that can integrate DNA into host genome thereby increase the yield of transactivator protein HBxAg that may deregulate many pathways involving in metabolism of cells. Several monogenic and polygenic risk factors are also involved in HCC development. This review summarizes the mechanism involved in HCC development and discusses some promising therapies to make HCC curative.

## 1. Introduction

HBV infection is a major health problem leading to significant death rate worldwide, especially in developing countries including Pakistan. HBV infects 350 million people worldwide, and 7–9 million in Pakistan [1]. HBV is a small enveloped DNA virus pertained to the hepadna family of viruses that integrates its DNA into the host genome and this integration of DNA is believed, in part, to be carcinogenic. Currently at least 10 HBV genotypes and several subtypes have been identified. Well-known genotypes are eight named as A to H [1]. The prevalence of specific genotypes varies geographically; genotype B is primarily found in south Asia with a unique genome structure [2] it was estimated that there is about 8% or <8% complete nucleotide sequence divergence in these genotypes [3, 4]. No differences in viral loads were found in relation to age, gender, or genotype in the African black HCC patients, whereas recent studies from Taiwan reported that HCC patients younger than 40 years of age had lower HBV DNA titre than older patients [5, 6]. HBV contains four overlapping reading frames (ORF): S, P, X and pre C. The SORF encodes the three viral surface proteins: the preS1 (or Large), the preS2 (or Middle) and the S (or small) that corresponds to HBsAg. The soluble antigen “e” (HBeAg) and (HBcAg) encoded by pre-C ORF. The viral polymerases possess DNA polymerase, reverse transcriptase and RNase H

activities, and terminal proteins are encoded by P ORF. The X ORF encodes the regulatory X protein, which is capable of transactivating the expression of numerous cellular and viral genes and vital for virus replication [7]. The HBsAg is the first serological marker detectable in serum, primarily appears during the incubation period of virus life cycle and rapidly rises in titer. Another core antigen HBeAg was reported to inhibit production of interleukin 6 (IL-6) through the suppression of nuclear factor kappa B (NF- $\kappa$ B) gene expression. It has been demonstrated that HBeAg could impair both adaptive and innate immune response in order to promote HBV infection. One study reported that the inflammatory cytokines such as IL (12A, 8, 6), IFN- $\alpha$ 1 and  $\beta$ , and TNF mRNA expression downregulated in HBeAg-positive hepatic cell. Thus, it can be concluded that HBeAg can modify the gene expression of tumor suppresser genes in HBV infected patients [8]. HBcAg is a sign of active viral replication. It has been found that agglomeration of naturally occurring mutations in particular segments of HBcAg may be related to the development of HCC. Other patent risk factors for HCC include chronic viral hepatitis, cirrhosis, nonalcoholic fatty liver disease, heavy alcoholism and some particular inherited metabolic conditions such as alpha-1-antitrypsin deficiency and hemochromatosis [9], oxidative stress, aflatoxin B1, drugs, medications, chemicals, and up/downregulation of several cellular and immune responsive genes, and so forth.

Expression analysis done by tissue microarray technique shows that if the expression of HBsAg, HBcAg, p21 and Rb proteins in HCCs is downregulated, the possibilities of HCC development will increase as these proteins are also tumor suppressors [10]. The present review will focus on the genetic causes of HCC; genes involved in the development of HCC and the role of HBx protein. Although vaccination is available but once a person gets infected with HBV, there is no treatment other than the supportive care.

## 2. Hepatitis Virus Genome Organization

The HBV genome has many features that distinguish it from other viral genomes for example, (a) circular DNA conformation (b) partially double-stranded DNA and (c) dependence on a reverse transcriptase. It has been concluded that dependence on reverse transcriptase helps in the persistence of viral infection in host hepatocytes. Genome size of HBV is about 3.2 kb having four open reading frames (ORF): S, C, P, and X. The ORF S encodes the viral envelope proteins (HBsAg) and has been divided into three main parts preS1, preS2, and S on the basis of structure and functions. The C gene encodes HBcAg and HBeAg. The C gene encodes a core and precore region. The product is HBeAg; if the translation begins from the precore region. If translation starts from core region then the product is HBcAg. The function of HBeAg needs more attention to get fully understood although most of the literature depicts that HBeAg promotes viral persistence in hepatocytes [11]. The largest protein of HBV is P, which encodes for DNA polymerase. The P ORF has been divided into three domains: (1) the reverse transcriptase (RT) domain (2) ribonuclease H domain and (3) terminal protein domain. The function of terminal protein domain is to initiate of minus strand synthesis and encapsidation. The HBV X ORF HBxAg whose main function is in carcinogenesis. Other functions of X gene are repairing of DNA, signal transduction, transcriptional gene activation and to stop protein degradation [12–14], and so forth. Human are natural host of HBV.

## 3. HBV Integrated DNA and Gene in Host Genome Causes HCCs

HBV DNA integration into host genome is a compelling step during CHB infection. Viral DNA integration disrupts the functioning of several genes which are important for normal cell growth and differentiation. The chance to get HCC by HBV is directly proportional to the number of random integrations of viral genome into host liver cells [15]. The integration of HBV DNA into hepatocytes is an integral step for persistent viral infection that leads to CHB infection, which ultimately causes HCC [16]. As viral DNA integration rearranges both host and viral genes leading to the production of altered protein products making hepatocytes more susceptible [17]. The insertion of viral genome results in chromosome deletions and other general genomic instability [18] that may activate several pathways switching on HCC development [19]. Studies revealed that HBx, hepatitis B spliced protein (HBSP) and truncated preS2/S gene, found more frequently

than other genes in infected cells. It was demonstrated that expression of HBV proteins have a direct effect on many cellular functions, and some of these gene products can promote malignant transformation in hepatocytes [20, 21]. It was studied that the prevalence of pre-S deletions was significantly higher in HCC patients [22]. It was also suggested in 2010 that there is a strong link between pre-S2 deletion and HCC development [23]. The truncated pre-S2/S of HBV virus induces increased cell proliferation and strong endoplasmic reticulum stress, which induces oxidative stress and DNA damage; ultimately leading to HCC development [24, 25]. The HBSP has been found more frequently as compared to other proteins in HBV infected patients. HBSP may account for the association with HCC [26]. HBSP has found to be involved in persistence of HBV infection, this function of HBSP should be counted as one of the dominating causes for HCC [27].

## 4. Role of HBx in HCC Development

Hepatitis B virus X (HBx) gene plays a central role in HBV-related HCC progression and stimulation [28]. By promoting the level of G1 protein, HBx protein affects the normal physiology of hepatocytes and cell cycle progression. It was studied that G1-promoting proteins act as activators for CDK4, requires for HBV replication [29]. Previous studies suggest that up to 50 thousand copies per cell [30]. Quiescent cells may also undergo cell cycle progression through the expression of HBx protein in infected cells [31]. Moreover, HBx acts as an activator for a huge range of viral promoters [32–34]. Thus HBx gene expression is of prime importance for viral reproduction within living cells [35]. Most studies demonstrated that HBx protein is mainly expressed in cytoplasm but may also be detectable in nucleus of infected cells [36–42]. It was disclosed by Chen and his colleagues that HBx protein through a Crm-1-dependent nuclear export pathway shuttles between the cytoplasm and nucleus. HBx protein enhances NF- $\kappa$ B localization to the nucleus, with subsequent activation of respective transcriptional pathways [43]. This ultimately results in the progression of HCC development.

## 5. Impact of HBx on the Regulation of Signaling Pathways

A group of scientists in Germany indicate that HBx is fundamentally present in the cytoplasm and stimulates protein kinase C and other proteins that are activated by stress stimulation, that is, Jun N-terminal kinase, Inhibitor Kappa B Kinase (IKK), Janus kinase/STAT, Phosphoinositide 3-kinase (PI-3-K) and protein kinase B/Akt. HBx protein can also be detectable in the powerhouse of infected cells, where it increases the expression of B-cell lymphoma-2 (Bcl-2) family [44] and increases the risk of HCC formation. Different studies indicate that HBx protein works in cooperation with cellular transcription factors and induces transformation [45] in liver cells causing tumors [46, 47] or cancer [48–50]. Thus, studies proved that expression of HBV X gene in hepatocytes is a positive hallmark of HCC. It was reported that bone morphogenetic protein (BMP-9) plays a crucial role in nonhepatic

tumors [51]. Whereas BMP4 and BMP7 were up-regulated in cirrhosis, BMP4 and BMP7 along with SRC were further up-regulated in hepatocellular carcinoma [52]. Thus, BMP is related to invasiveness of HCC (Table 1).

## 6. HBx and Transcription Regulation

A rapid cytoplasmic signaling cascade initiated by activated Ras proteins. As HBx protein strongly elevate the levels of phosphorylated Raf, GTP-bound Ras and tyrosine-phosphorylated and activates MAP kinase [53] that eventually leads to HCC formation in hepatocytes. In another study, it is found that HBx can increase calcium level in mitochondria and promote store-operated  $Ca^{2+}$  entry (SOCE) to maintain higher cytosolic calcium level that stimulate HBV replication [54]. HBx can prompt cell cycle regulatory pathways and apoptosis [55]. HBx is found to be linked with many other components of the basic transcriptional machinery, including transcription factor II B (TFIIB), Transcription factor II H (TFIIH), the TATA-binding protein (TBP) and the RPB5 subunit of RNA polymerases [56–58]. HBx strongly mediates apoptosis in certain types of cells. HBx has the ability to inhibit the pRb tumor suppressor function and increase E2F transcription factor1 activity that is a positive cell cycle regulator [59]. Moreover, HBX protein can obstruct cell death mediated by p53, TNF, Fas and TGF- $\beta$  [60, 61]. The HBx can activates the Jak-STAT signaling pathway by acting as an inducer [62] it also breakthrough the liver cancer by down-regulating the dual-specificity protein phosphatase (PTEN) and Phosphatidylinositol 3,4,5-trisphosphate 3-phosphatase, known as a tumor suppressor genes [63]. The HBx protein can provoke the activities of the PI-3K-AKT/PKB, ERK. It was found that matrix metalloproteinase 9 (MMP-9) expression also enhanced by dual transcriptional upregulations of AP-1 and NF- $\kappa$ B [64]. Studies revealed that ribosomal protein S3a (RPS3a) interacts with HBx, and contributes to viral induced oncogenesis by enhancing HBx-induced NF- $\kappa$ B signaling pathway that results in HCC development [65]. HBx has been variably reported to activate *STAT3*, *WNT/CTNNB1* or bind to and inactivate the TP53 protein [66, 67]. HBx inhibits p53-mediated transcriptional activation and contributes to the molecular pathogenesis of human HCC [68]. Other protein targets for HBx are damaged DNA binding proteins, p53, proteasome subunits; these proteins interacts with the cyclic AMP-responsive element, ATF-2 and basal transcription factors [69]. It has been found that during HBx-induced-HCC many cellular cytoskeletal genes such as microtubule genes tubulinb 2, tubulinb 3, tubulinb 6, keratin 8 (K-8) and keratin 18 (K-18), actin1 (Actg1) and intermediate filament genes periplakin were dysregulated, As it has been documented that these genes were closely clustered and up regulated in liver tissues [70]. The metastasis-associated protein 1 (MTA1) gene is one of the important transcriptional target of HBx protein; It has been found that MTA1 activates hypoxia-inducible factor  $1\alpha$  and vascular endothelial growth factor which contributes to angiogenesis in hepatic cancer [71]. The MTA1 gene expression found to be significantly higher in HCC [72]. The HBx protein may increase telomerase reverse transcriptase

(TERT) expression as well as telomerase activity. The p21-activated kinase (PAK1) also increase the life-span of hepatocytes and contributing to malignant transformation as well as promote tumor growth [73]. Thus; several transcriptional regulatory genes are activated by the interaction with HBx protein that can eventually lead to HCC (Figure 1).

## 7. Monogenic and Polygenic Risk Factors That Induced HCC

Primary liver cancer is frequently cause by HBV induced HCC. This form of cancer is different from other forms of hepatic carcinomas. Vaccination is used to prevent HBV induced HCC, but vaccination does not protect those already infected with HBV. Most common risk factors for developing HCC are; alcoholic liver disease, and nonalcoholic steatohepatitis (NASH) [74] intake of aflatoxin contaminated food, diabetes, obesity, certain hereditary conditions such as hemochromatosis, and some metabolic disorders [75–77]. It was found that rare monogenic syndromes, alpha1-antitrypsin (AAT) deficiency and glycogen storage disease type 1 is caused by several mutations in a gene named *SERPINA1*. This gene was found to encode a serine protease inhibitor. Studies also revealed that over expression of *SERPINA1* in liver causes the inhibition of neutrophils elastase. Over expression of *SERPINA1* in liver causes the inhibition of neutrophils elastase. Moreover, *SERPINA1* also caused glycogen storage disease type 1. Hemochromatosis gene (HFE) is inherited as an autosomal recessive trait. This gene is the cause of iron over load in liver. Both glycogen and iron over load increase the chance of HCC progression. Other known monogenic factors that involve in HCC development are acute intermittent hepatic porphyria (AIP), fumarylaceto acetate hydrolase (FAH) as well as hereditary tyrosinemia type I and Familial porphyria cutanea tarda (PCT). It was found that children with tyrosinemia are at high risk of liver transplantation as beyond age of two years the incidence of HCC increases substantially [78]. A couple of familiar conditions or diseases inherited as polygenic traits, for example, autoimmune hepatitis (AIH), hypothyroidism and type 2 diabetes may also contribute to HCC development [79]. The genetic heterogeneity that is cause by a number of unlinked single gene defects may increase the susceptibility to HCC [80]. It has been found that among men with diabetes, the risk of chronic nonalcoholic liver disease and HCC is doubled [81]. Diabetes mellitus is an independent factor linked with HBV induced HCC [82]. Other factors that were found are obesity, the nonalcoholic fatty liver Disease (NAFLD). Obesity can lead to insulin resistance (IR) and steatosis, both these factors are closely linked with excretion of inflammatory cytokines. Therefore, diabetes and obesity can cause hepatic inflammation and oxidative stress resulting in hepatocyte's injury, subsequently HCC. IL6 and TNF production also up regulated during obesity induced HCC. Thus linking liver steatosis, with inflammation and expression of oncogenic transcription factor *STAT3*, a phenotype was shared by both virus and nonvirus-related HCCs [83]. It was revealed that Hepatoma-derived growth factor (HDGF) expressed more strongly in the cytoplasm and

TABLE 1: Genes upregulated during HBV-induced Hepatocellular Carcinoma

Genes up-regulated during HCC				
Gene name	Gene	Function	Activation by	Reference
B-cell lymphoma/leukemia 2	BCL2	Apoptosis-related genes	HBV pre-S2 increased Bcl-2 expression	[86]
Baculoviral IAP repeat containing 5 (survivin)	BIRC5	Cell cycle, regulation, apoptosis inhibitor	HBV and HCV	[87]
Cyclin D1	CCND1	Regulators of CDK kinases, interact with tumor suppressor gene	HBV	[88]
CASP8 and FADD-like apoptosis regulator	CFLAR	Apoptosis-related genes	HBV-induced HCC	[89]
Type II keratin Kb8	<i>KRT8</i>	Cytoskeletal organization, cirrhosis	HBV	[89]
Ribosomal protein S5	<i>RPS5</i>	Protein synthesis	HBV	[89]
Insulin-like growth factor binding protein 2	<i>IGFBP2</i>	Cell membrane receptor related genes	HBV	[89]
Matrix metalloproteinase 9	<i>MMP9</i>	Metastasis-related genes	HBV	[89]
ATP synthase F 1	<i>ATP5F1</i>	Transportion	HBV	[89]
Frizzled-7 receptor	FZD7	Activates the Wnt/beta-catenin pathway	HBV	[90]
Insulin-like growth factor2	IGF2	Stimulatory role, gestation	HBV	[91]
Maternally-transmitted human gene	<i>H19</i>	Cancer causing	HBV	[91]
Tumor growth factor beta 1	TGFB1	Tumor suppressor	HBV	[92]
Induced myeloid leukemia cell differentiation protein	Mcl-1	Controlling life and death decisions in response to rapidly changing environmental cues and immune response	HBV pre-S2Δ protein	[86]
Transforming growth factor alpha	TGFA	Morphogenesis	HBV	[93]
Lymphoid enhancer-binding factor 1	LEF1	Regulatory proteins and potential drug target	Chronic HBV	[94]
Nuclear factor kappa B	NFKB1	Inflammation, immunity, differentiation, tumorigenesis, and apoptosis	HBV	[95]
Insulin receptor substrate 1	IRS1		HBV	[96]
Peptidyl-prolyl cis-trans isomerase NIMA-interacting 1	PIN1	Cell proliferation, cell survival, immune responses	HBV	[97]
Hepatocyte growth factor receptor (HGFR)	MET	Protooncogene, angiogenesis, gastrulation,	HBV	[98]
Protein tyrosine kinase 2	PTK2	regulation of the tumor suppressor p53	HBV and HCV	[99]
Ras-related C3 botulinum toxin substrate 1	RAC1	Cell growth, cytoskeletal, activation of protein kinases	HBV	[91]
Ras homolog gene family, member A	RHOA	Regulation and timing of cell division.	HBV	[91]
Mothers against decapentaplegic homolog 7	SMAD7	Overexpression causes cancer	HBV	[100]
Immunoglobulin transcription factor 2	TCF4	Immune response	HBV	[101]
TNF-related apoptosis-inducing ligand (TRAIL)	TNFSF10	Activates NFkappaB	HBV	[102]
X-linked inhibitor of apoptosis protein	XIAP	Inhibits caspase 3, 7, and 9	HBV	[103]
Proto-oncogene tyrosine-protein kinase	SRC	Regulation of embryonic development and cell growth.	HBV	[47]

TABLE I: Continued.

Gene name	Genes up-regulated during HCC		Activation by	Reference
	Gene	Function		
Store-operated Ca(2+) entry	SOCE	Preventing the overload of the cell with excessive Ca(2+) ions	HBx	[104]
Metastasis associated 1	MTA	Contributes to angiogenesis	HBV	[68]
BAX	Upregulated by HBx	Apoptotic activity	Bcl-2-associated X protein	[105]

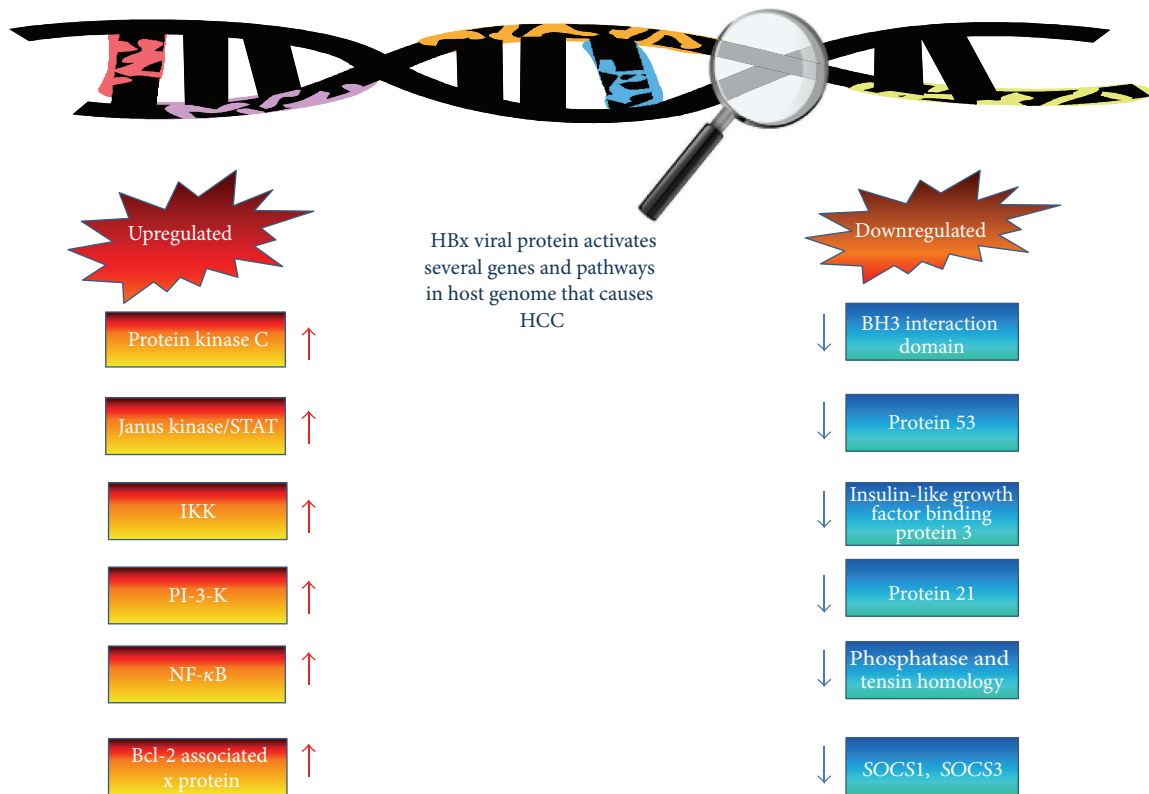


FIGURE 1: HBx protein activated Pathways and Genes.

nucleus of HCC cells as compared to other normal hepatocytes. High expression of HDGF is one of the possible causes of HCC progression [84]. Hereditary hemochromatosis (HH) is concord with an increased risk for HCC. The risk for HCC also increased among the patients of homozygous  $\beta$  thalassemia and African iron overload disease [85]. All these monogenic and polygenic factors result in HCC development as depicted in Figure 2.

### 8. Pathways Activated by HBV Infection

Many pathways of cellular immune system are activated during HBV infection. The deregulation of signaling pathways including MAPKs, p53, Sex steroid, Wnt/ $\beta$ -catenin, transforming growth factor  $\beta$  (TGF $\beta$ ), PI3K/AKT, cytokines, IKK/NF- $\kappa$ B and Hedgehog (Hh) were found to be closely related with HCC development. These signaling cascades

mostly leads to down-regulation of tumor-suppressor gene and up regulation of tumor-causing genes [120]. It has been studied that both Cytokine lymphotoxin (LT)  $\alpha$  and  $\beta$  and their receptor (LT $\beta$ R) are up regulated in HBV-induced HCC. Sustained LT signaling is another channel involved in HBV-induced HCC [121]. Many signal transduction process that were important for stem cell differentiations and proliferation also deregulated during hepatocarcinogenesis (Table 2) [122]. In hepatocellular carcinoma chromo-domain helicase/ATPase DNA binding protein 1-like gene (CHDIL) was frequently amplified. The CHDIL involves in metastasis by increasing cell motility, tumor cell migration, epithelial-mesenchymal transition (EMT) via ARHGEF9-mediated Cdc42 activation, invasion and inducing filopodia formation. The results obtained indicates that CHDIL-ARHGEF9-Cdc42-EMT might be a novel pathway involved in metastasis and HCC progression [123]. The level of IL6 was found to be

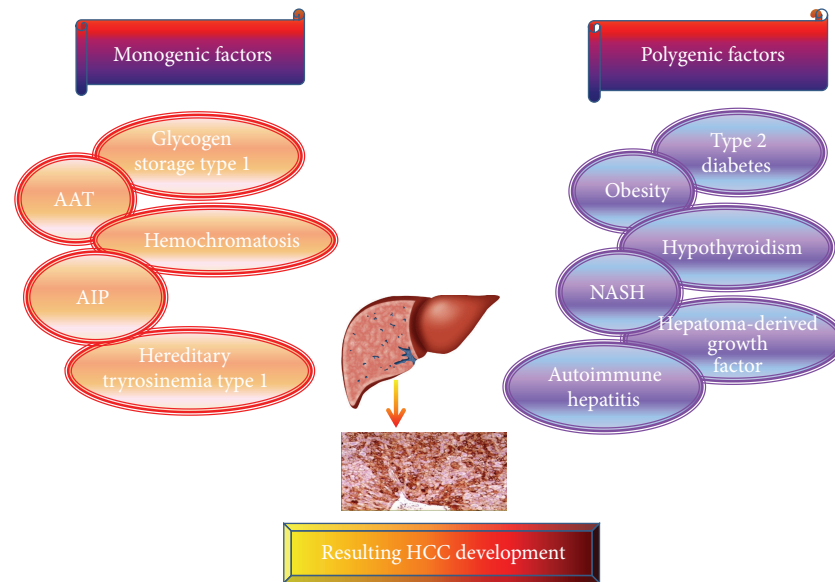


FIGURE 2: Monogenic and Polygenic diseases causing HCC development.

TABLE 2: Genes down regulated during HBV induced hepatocellular carcinoma

Genes Down regulated during HCC:				
Gene	Gene name	Activated by	Function	Reference
BID	BH3 interacting domain	HBx protein	Cell death regulation	[106]
P53	Protein 53	HBx	Tumor suppressor	[107]
p21WAF1	Protein 21	HBx	Stress response, acts with p53	[107]
IGFBP3	Insulin-like growth factor-binding protein 3	HBx		[107]
CASP8	Caspase 8	HBV	Apoptosis-related cysteine peptidase	[89]
CDKN1A/p21	Cyclin-dependent kinase inhibitor 1A	HBV	Inhibits the activity of cyclin-CDK2	[107]
DLC1	Deleted in liver cancer 1	HBV	Tumor suppressor, cell growth, and proliferation	[108]
FADD	Fas-associated protein with death domain	HBV	Adaptor molecule that bridges the Fas-receptor	[109]
IGFBP1	Insulin-like growth factor-binding protein 1	HBV	Tumor suppressor	[110]
ITGB1	Integrins beta 1	HBV	Embryogenesis, hemostasis, tissue repair, immune response	[111]
Hhip	Hh-interacting protein	HBV	Modulates hedgehog signaling	[112]
PTEN		HBV	Apoptosis, cell movement	[113]
RBI	Retinoblastoma 1	HBV	Oncogenic, tumor suppressor	[114]
SMAD4		HBV	Transmitting chemical signal, regulates cell growth and division	[115]
SOCS1	Suppressor of cytokine signaling 1	HBV	Negative regulator in TNF-induced inflammation and activation of c-jun N-terminal kinase	[116]
SOCS3	Suppressor of cytokine signaling 3	HBV		[117]
TGFBR2	Transforming growth factor, beta receptor II	HBV	Transmits signals, stimulation of cell growth and division, differentiation	[118]
CDKN2A	Cyclin-dependent kinase inhibitor 2A	HBV and HCV	Cell cycle control, tumor suppressor gene	[119]

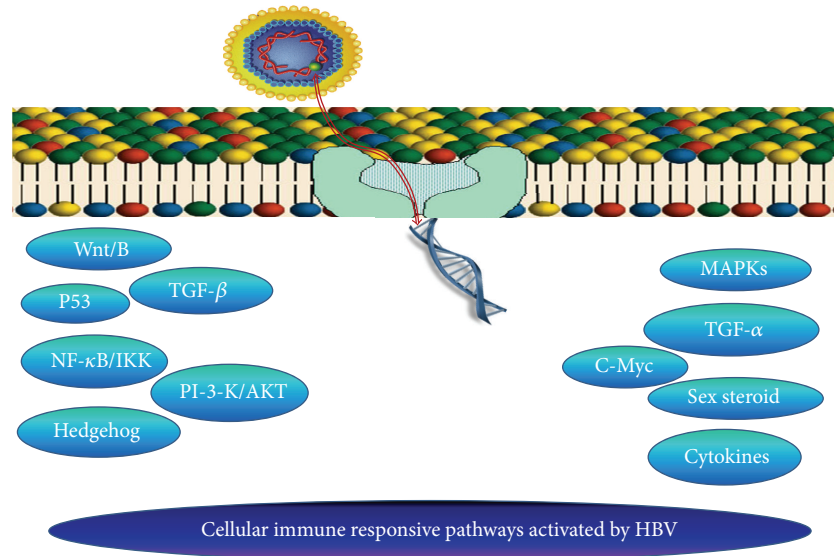


FIGURE 3: Pathways involve in HBV induced HCC.

increased in HCC cells which proved that IL6 and Inflammatory cytokines, play a significant role is HCC development [51]. Level of IL6 may also predict the shift from viral hepatitis to HCC in humans [124] due to Hh signal activation. It has been documented that the expression of HBx and Hh is highly correlated in human liver cancer cell lines [125]. It was studied that in patients with HBV induced HCC, transforming growth factorbeta (TGF- $\beta$ ) cytokine and its isoforms initiates a signaling cascade, which is closely linked to liver cirrhosis and fibrosis. A scientist found that when HBx is over expressed in hepatic cell line then tuberous sclerosis complex 1 (TSC1), I $\kappa$ B kinase  $\beta$  (IKK $\beta$ ) and mammalian target of rapamycin (mTOR) downstream effector S6 kinase (S6K1) signaling pathways upregulated. Thus, through the use of IKK $\beta$ , HBx deregulates TSC1/mTOR signaling, which is closely linked to HBV-associated HCC development [126]. C-Myc and TGF- $\alpha$  were found to induce continued and cumulative transcriptional changes in the liver and starts oncogenesis [127]. One study showed that LSF is a key mediator of the Notch signaling pathway [128]. One group of scientists in China disclosed that transcriptional inactivation of p16 and p15 genes also involves in hepatocarcinogenesis. The p15 and p16 genes inactivation may be caused by 5' CpG Island methylation in primary HCC [129]. It has been found that three dominant group of genes that were up regulated by HBV heat shock proteins, oxidative and metabolic stress and growth and apoptosis-related genes [130]. The vimentin and IQGAP1 mRNA expression levels increased significantly throughout hepatotumorigenesis provide another target to treat HCC [131]. Targeting the key molecules in the oncogenic signaling pathway might be a promising strategy for HCC therapy (Figure 3).

### 9. Oxidative Stress Induced HCC by HBV

As HBV is a DNA virus which integrates its genome inside the host genome, during HBV infection, viral replication occurs

inside infected hepatocytes within viral capsids. In this manner, viral genome conceals itself from pattern recognition receptors (PRRs), of innate immune system, preventing the detection of initial HBV infectious particles [132] PRRs including Toll-like receptors (TLRs) [133, 134] that recognize the pathogen-associated molecular patterns leading to an alter macrophage phenotype. These macrophages secrete reactive oxygen species (ROS); such as type I IFNs (IFN- $\alpha$  and IFN- $\beta$ ), nitric oxide and other cytokines and chemokines, which may results in up-regulation of intracellular response elements like IRFs, iNOS, NO. It was studied that the general production of nitric oxide and ROS by activated macrophages may also cause hepatocytes destruction [135]. ROS can cause oxidative protein, DNA damage. Oxidative damage to tumor suppressor genes [136]. ROS also effects the central cellular processes such as apoptosis and proliferation leading to the development of cancer [137]. Chronic HBV infection results in increase intracellular iron level in liver. The presence of HBV surface antigen and iron deposition strongly linked with each other [138]. In chronic HBV infected patients, TNF- $\alpha$  also boost up causing inflammation in hepatocytes [139]. It has been stated that viral infection induces oxidative stress affect the cellular protein kinase/phosphatase balance, which has been studied in a number of tumors. The reactive oxygen species has direct effects on major cellular processes through the activation of transcription factors, including NF- $\kappa$ B, MAPK and AP-1 pathways [140]. Up regulation of AP-1 results in enhanced cyclin D1 expression and CDKs, known to promote cell division by mitosis. It was narrated that reactive oxygen species activates the NF- $\kappa$ B by cytokines and TNF. Genes that were up-regulated during oxidative stress are aldo-keto reductase 1 family B7 (AKR1B7-) like protein gene, hemoglobin alpha (HBA1) and beta (HBB) [141, 142] Oxidative Stress responsive Genes APOE, ATOX1, and CAT [143-145] were found to be activated during infection. Genes that involve in DNA damage are CCND1, CDKN1A (p21CIP1/WAF1), MSH2, MSH3,

TP53, and XIAP [146, 147], along with modified gene expression and mutations are all required participants in the process of carcinogenesis. It was found that oxidative stress is associated with hepatitis B activity and XRCC1 gene is putatively associated with DNA damage [148]. Although all these events derived by different gene products, a common theme which is the entanglement of ROS that cause oxidative stress. It was studied that CD8 T-cells effectively control HBV replication, especially in the presence of IFN-gamma and TNF-alpha [149]. The platelets Causes hepatic injury by promoting accumulation of virus-specific CD8 (+) T cells. Previous data about CD8<sup>+</sup> shows that although it works to control HBV viral particle clearance and replication, but it may also cause liver injury by recruiting nonviral specific T-cells [150]. As studies revealed that immune mediated necro-inflammation is another cause of HCC development [151]. It was found that CD8<sup>+</sup> T cell causes hepatic inflammation derive by INF gamma [152]. It was demonstrated that metabolic syndrome (MS) was associated with both cryptogenic cirrhosis and nonalcoholic steatohepatitis, Currently MS can be consider as an independent and major risk factor for CHB infection related Cirrhosis [153–155] and HCC development [156]. The risk of HCC was found positively correlated with the number of MS components found in patients not chronically infected with HBV [157].

## 10. Role of cccDNA in HBV-Related HCC Recurrence

It has been exposed by a team of scientist that HBV cccDNA is a unique intermediate that serve as a template for the production of HBV pregenomic RNA (pgRNA) that in return responsible for the persistent HBV infection in hepatocytes. It was found that any piece of pgRNA can be act as a template for minus DNA strand synthesis [158–160]. The cccDNA level increases unexpectedly in the initial phase of proliferation after that the level of cccDNA decreased dramatically in the cells during cell division due to loss of extra-chromosomal plasmid DNA [161] thus cccDNA level shows dynamic expression in different phases of cell growth. The pegylated interferon alpha-2b (peg-IFN) and adefovir dipivoxil (ADV) antiviral therapy led to considerably decreased the cccDNA levels by a primarily noncytolytic mechanism [158, 162]. The peginterferon alpha-2b, lamivudine treatment also results in decrease level of HBsAg that is correlated with cccDNA level [163]. Some patients also treated with tenofovir disoproxil fumarate (TDF) which shows incomplete or low response to ADV therapy [164]. A positive correlation was found between cccDNA and HBcrAg at the incidence of HCC reoccurrence, thus cccDNA is a positive haalmark of HBV related HCC reoccurrence [165]. It was reported in ScienceDaily that IFN $\alpha$  can cause silencing of cccDNA as well as Lymphotoxin beta receptor (LTbR) agonisation and also can provide novel alternative therapeutic approach for curing CHB infection [166].

## 11. Promising Therapies for HBV Induced HCC

*11.1. Diagnosis.* HCC due to HBV is a lethal disease as the diagnosis is late in this type of cancer, best method, untill now for the diagnosis of HCC is computed tomography (CT) of the liver, Magnetic resonance imaging (MRI) with contrast enhancement is most common method for detecting liver inflammation. Liver angiography with lipiodol injection and follow by CT is another accurate method for detecting liver lesions. Liver biopsy is seldom required for diagnosis.

## 12. Antiviral and Nonsurgical Treatments for HCC

Effective antiviral therapies are now available. IFN- $\alpha$  therapy was proven to be efficient in reducing the risk of HCC recurrence patients with small HCC [167]. Continued suppression of HBV replication with anti-nucleoside or anti-nucleotide or by analogs may decrease the risk of HBV-related HCC development; it was found that Long-term lamivudine treatment can prevent complications of HBV-related liver disease. However, when lamivudine does not affect than entecavir and Adefovir dipivoxil have been shown to be more effective and safe for the treatment of patients with Chronic hepatitis [168]. Patients who developed adefovir resistance will respond only to adefovir monotherapy rather than lamivudine and adefovir combination therapy [169]. Angiogenesis inhibition is a natural therapeutic target for all solid tumors, targeted agent sorafenib provided proof-of-concept for molecularly targeted therapy in advanced HCC, tyrosine kinase inhibitor (TKIs) such as brivanib, everolimus, and monoclonal antibodies (e.g., ramucirumab) erlotinib, sunitinib, vandetanib, cediranib, brivanib, foretinib, and dovitinib [170] are being tested as second-line therapies [171], brivanib is a dual fibroblast growth factor pathway and VEGF receptor inhibitor, additional agent for the treatment of patients with HCC includes, ramucirumab, ABT-869, bevacizumab, ARQ-197 and everolimus [172]. The early evidence of anti-tumor activity was shown by sunitinib. Despite significant progress, progressive HCC remains an incurable disease; combination of molecular targeted approach will be helpful in management of advanced disease. Transarterial chemoembolization has been tested and found significantly important in increasing survival, in highly selected patients with good liver function [173]. Hormonal therapy and biotherapy, radiotherapy, thermal and laser ablation, percutaneous alcohol injection, cryoablation, as well as radiofrequency ablation (RFA) percutaneous ethanol injection (PEI) and systemic therapy are also potentially effective curative [174, 175].

## 13. Recent Advancement in HBV Research

Recently, in a new study by Alasdair Steven (Chief of the NIAMS Laboratory of Structural Biology Research) and Paul Wingfield (Chief of the NIAMS Protein Expression Laboratory) a unique antibody that stably binds e-antigen was



developed. Understanding the structure of e-antigen will be helpful in future studies. As this antigen plays a significant role in HBV persistence. It was elucidated that full surface vaccines gives stronger immunity and are good even for neonates [176].

## 14. Conclusion

In conclusion, the present review indicates that HBV infection is in one of the major cause of HCC development. HBV protein S, C, P and X are responsible for viral replication and activation of several cell signaling pathways such as NF- $\kappa$ B, PI-3K and Hedgehog and eventually lead to HCC. Moreover, a couple of polygenic and monogenic pathways such as NASH, Obesity, and Type 2 Diabetes and Glycogen Storage disease type 1, Hemochromatosis respectively were also involved in HCC development. Targeting the key element involves in different signaling pathways that are activated by HBV infection and genetic factors will be helpful in future to find the easy and permanent cure from HCC. Moreover, the information regarding up-regulation and downregulation of genes during HCC will be helpful to find noninvasive markers in blood.

## Conflicts of Interests

All authors have no kind of institutional or financial conflict of interests.

## Authors' Contribution

The first two authors contributed equally in this paper.

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