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3
Dissecting novel mechanisms of hepatitis B virus related hepatocellular carcinoma
using meta-analysis of public data

Aljabban J *et al.* Dissecting novel mechanisms of HBV HCC

Abstract

BACKGROUND

Hepatitis B virus (HBV) is a cause of hepatocellular carcinoma (HCC). Interestingly, this process is not necessarily mediated through cirrhosis and may in fact involve oncogenic processes. Prior studies have suggested specific oncogenic gene expression pathways were affected by viral regulatory proteins. Thus, identifying these genes and associated pathways could highlight predictive factors for HCC transformation, and has implications in early diagnosis and treatment.

AIM

To elucidate HBV oncogenesis in HCC and identify potential therapeutic targets.

METHODS

We employed our Search, Tag, Analyze, Resource platform to conduct a meta-analysis of public data from National Center for Biotechnology Information's Gene Expression Omnibus. We performed meta-analysis consisting of 155 tumor samples compared against 185 adjacent non-tumor samples and analyzed results with ingenuity pathway analysis.

RESULTS

Our analysis revealed liver X receptors/retinoid X receptor (RXR) activation and farnesoid X receptor/RXR activation as top canonical pathways amongst others. Top upstream regulators identified included the Ras family gene rab-like protein 6 (RABL6). The role of RABL6 in oncogenesis is beginning to unfold but its specific role in HBV-related HCC remains undefined. Our causal analysis suggests RABL6 mediates pathogenesis of HBV-related HCC through promotion of genes related to cell division, epigenetic regulation, and Akt signaling. We conducted survival analysis that demonstrated increased mortality with higher RABL6 expression. Additionally, homeobox A10 (HOXA10) was a top upstream regulator and was strongly upregulated

in our analysis. HOXA10 has recently been demonstrated to contribute to HCC pathogenesis *in vitro*. Our causal analysis suggests an *in vivo* role through downregulation of tumor suppressors and other mechanisms.

CONCLUSION

This meta-analysis describes possible roles of RABL6 and HOXA10 in the pathogenesis of HBV-related HCC. RABL6 and HOXA10 represent potential therapeutic targets and warrant further investigation.

Key Words: Hepatitis B virus; Hepatocellular carcinoma; Genomics; Meta-analysis

Aljabban J, Rohr M, Syed S, Cohen E, Hashi N, Syed S, Khorfan K, Aljabban H, Borkowski V, Segal M, Mukhtar M, Mohammed M, Boateng E, Nemer M, Panahiazar M, Hadley D, Jalil S, Mumtaz K. Dissecting novel mechanisms of hepatitis B virus related hepatocellular carcinoma using meta-analysis of public data. *World J Gastrointest Oncol* 2022; In press

Core Tip: Hepatitis B virus (HBV) is a cause of hepatocellular carcinoma (HCC). Interestingly, this process is not necessarily mediated through cirrhosis and may in fact involve oncogenic processes. Prior studies have suggested specific oncogenic gene expression pathways were affected by viral regulatory proteins. Thus, identifying these genes and associated pathways could highlight predictive factors for HCC transformation, and has implications in early diagnosis and treatment. Our manuscript leverages big data to offer key insights to oncogenesis of HBV infection in HCC. We were able to dissect key genetic drivers to disease and namely demonstrate a newfound role for rab-like protein 6 and homeobox A10.

INTRODUCTION

Hepatitis B virus (HBV) is a major cause of liver disease, significantly contributing to global morbidity and mortality. Recent estimates show there are approximately 240-300 million people chronically infected with HBV worldwide^[1,2]. Chronic HBV infection leads to cirrhosis in 30% of patients, of which 53% later develop hepatocellular carcinoma (HCC)^[3]. The Global Burden of Disease Study estimated that HBV-related cirrhosis and liver cancer annually causes 786000 deaths worldwide^[4]. HBV utilizes both direct and indirect means to promote HCC. For example, HBV-induced HCC, without cirrhosis suggests involvement of oncogenic pathways independent of chronic liver inflammation. Some studies have implicated viral regulatory proteins such as HBV X protein affecting gene expression pathways^[5]. In addition, mutations resulting in growth advantages may be conferred to infected cells by virtue of host chromosomal HBV DNA integration - a phenomenon known as insertional mutagenesis^[5]. In the decades since Garcia *et al*^[6] and Wang *et al*^[7] identified cyclin (CCN) A and retinoic acid receptor-beta genes, respectively, as targets of HBV integration, other points of vulnerability have been identified. Li *et al*^[8] found HBV integration into telomerase reverse transcriptase-promoter genes results in sex hormone-dependent responsiveness, providing a possible explanation for the threefold male-to-female preponderance of HBV-related HCC^[6-8].

Despite advancements in nucleoside nucleotide analog (NA)-based treatment, and adequate viral suppression resulting in undetectable HBV DNA, patients are still at risk for developing HCC. This may be due to NAs ability to suppress viremia but not eliminate infection and leading to oncogenesis. Importantly, chronic HBV infection, irrespective of cirrhosis represents a lifetime risk for HCC development 10-25 times greater than non-infected persons^[1,5,9]. These data highlight the importance of identifying predictive factors for HCC transformation, non-resolving acute infections, and chronic disease development. Understanding the evolution of HCC following HBV infection, and genetic signatures of HBV oncogenicity, will pave the way for improved risk assessment, treatment options, and patient outcomes. In this meta-analysis, we aim

to identify transcriptomic correlates of HCC development in patients with HBV infection.

MATERIALS AND METHODS

Search Tag Analyze Resource

We developed the Search Tag Analyze Resource (STARGEO) platform to utilize the wealth of genomics data featured in the National Center for Biotechnology Information's Gene Expression Omnibus. STARGEO allows for meta-analysis of transcriptomic signatures between sample sets, such as between disease and normal tissue, through tagging of biological samples from public data. Briefly, through stargeo.org, we searched for studies that studied HBV-related HCC. We then manually curated samples through the "Tagging" interface built into STARGEO based on interactive regular expressions. We gathered liver tumor samples under the "HBV_HCC" tag and control samples from adjacent tumor samples under the "HCC_Control" tag. More information on STARGEO can be found in our previous paper^[101]. To investigate HBV-specific HCC, we tagged 155 tumor and 185 adjacent non-tumor samples as a control. Samples were paired 1:1 within each study. Data was sourced from the GSE19665, GSE44074, GSE55092, GSE62232, and GSE67764 series^[102-106]. HCC patients in these studies had confirmed chronic HBV infection with no other co-morbid hepatic infections and had biopsies taken at time of diagnosis. Genetic analysis focused on hepatocytes. Stargeo.org mappings are based on mygene.info gene annotation service to map all probe identifiers to Entrez gene identifiers^[107]. The mean difference of contrasts for expressions and the standard deviation of that mean difference were calculated for each gene in every study. Standard meta-analysis with fixed and random effects model were used to combine these estimates across studies to generate both meta *P*-values and effects size across studies. Study weight percentages were calculated using the inverse variance method *via* the DerSimonian-Laird estimate^[108]. We use Python to achieve the analyses explained above in stargeo.org. More information on this particular analysis of HBV-related HCC can be found on

<http://stargeo.org/analysis/669/>. Individual genes can be searched and the number of patient samples in which we observed change in genetic expression is available, along with other information (see Figure 1).

Ingenuity pathway analysis

In order to dissect potential mechanism of disease, potential biomarkers, and therapeutic targets, we extracted more than 21000 genes for our meta-analysis and analyzed the output using the ingenuity pathway analysis (IPA) tool^[17]. Analysis was restricted to genes that showed statistical significance ($P < 0.05$) in both fixed and random effects models with an absolute experimental log ratio greater than 0.7 between experimental (HCC) and control samples. A total of 1035 genes were included in the IPA analysis. Top up- and downregulated genes determined by STARGEO are featured in Table 1. Genes analyzed by IPA are summarized in Supplementary Table 1 with P -values and experimental log ratios.

HCC is a complex disease that is a result of several pathological drivers. We used IPA Upstream Regulator analysis to elucidate upstream transcription regulators that best reflect our observed genetic expression dataset^[17]. The P -values are based on the degree of overlap of known effector targets and our gene list submission. The activation z-score illustrates the upstream regulator activation state, the magnitude of which represents likely activation states of upstream regulators.

Survival analysis

The association between rab-like protein 6 (RABL6) expression in HCC patients and survival was found using GPEIA2^[56]. GPEIA2 uses samples from The Cancer Genome Atlas (TCGA) HCC cohort and analyzes survival based on the Kaplan-Meier survival method. Expression was split into the top 75% and bottom 25% of RABL6 expression. There were no interactions with human subjects or interventions involved in this study. Additionally, as all content presented is sourced from publicly available data and no

patient-protected information was used. Therefore, no institutional review board approval was deemed necessary.

RESULTS

Top canonical pathways and gene candidates from HBV-HCC analysis

IPA analysis demonstrated liver X receptor (LXR)/retinoid x receptor (RXR) activation, lipopolysaccharide-interleukin-1 (LPS-IL-1) mediated RXR inhibition, acetone degradation, melatonin degradation, and farnesoid X receptor (FXR)/RXR activation as top canonical pathways in HBV-associated HCC (see Supplementary Table 2 for more information on top canonical pathways). FXR (NR1H4) was downregulated in our analysis suggesting its inhibition (Supplementary Table 2).

Top upregulated genes in our analysis are implicated in several signaling processes (Table 1). For example, glypican-3 (GPC3), a cell surface glycoprotein that interacts with Wnt/ β -catenin, Yes associated protein, and Hedgehog pathways^[10]. Additionally, Akt signaling was activated through the epidermal growth factor receptor kinase substrate 8-like protein 3, a substrate for the epidermal growth factor receptor^[11]. Furthermore, we found upregulation of the serine/threonine kinase PDZ-binding kinase (PBK), a mitotic kinase related to mitogen-activated protein kinase kinase (MAPKK)^[12]. Other top regulated genes are involved in cell cycle division and control including anilin, CCNB1, assembly factor for microtubules, cyclin dependent kinase 1, and protein regulator of cytokinesis 1. Moreover, we found upregulation of the serine/threonine kinase NIMA related kinase a mediator of centrosome separation in mitosis and meiosis, and deniticleless, an adaptor of the E3-ubiquitin ligase that targets p21 to drive cell division^[13].

Top downregulated genes are involved in liver metabolism and other processes. Several members of the C-type lectin family CLEC4G, CLEC1B, and CLEC4M were found among the most repressed genes. C-type lectins participate in adhesion and can act as signaling receptors for inflammation and immune-related processes^[14]. Other downregulated genes are indicative of impaired liver function including the liver

specific anion transporter solute carrier organic anion transporter family member 1B3, iron regulator hepcidin (HAMP), and the metallothionein (MT1M)^[15]. Lastly, we found downregulation of Hh-interacting protein (HHIP), a negative regulator of Hedgehog signaling^[16].

Network analysis of HBV-HCC

To elucidate top disease functions from our results we employed ¹ the IPA Network analysis function^[17]. IPA ranks networks from the Global Molecular Network based on the number of focus genes from given networks that match with our analysis. Significance is given by the p-score [$p\text{-score} = -\log_{10}(P\text{-value})$]. We identified 25 networks with most being involved in cancer, cell cycle, gastrointestinal disease, and other disease functions. The top 6 networks are summarized in Table 2 and the top network is illustrated in Figure 2 (see Supplementary Table 3 for all networks).

Analysis of potential drivers of HBV-HCC pathogenesis

To investigate genes that most greatly influenced our gene dataset and oncogenesis we used IPA to identify top upstream regulators (see methods). We searched for genes that were identified as the most activated up-regulators in IPA and were upregulated in our dataset (see Supplementary Table 4). The most activated upstream regulator was RABL6, a member of the Ras family of GTPases^[18]. We also found activation of transcription factors T-box transcription factor 2 (TBX2) involved in hepatocyte proliferation, migration, and invasion^[19]. We also noted activation of E2F transcription factor 1 (E2F1), which has a mixed but predominantly proliferative role in HCC^[20]. Additionally, we found activation of the transcription factor forkhead box M1 (FOXO1), which promotes cell turnover through CCNB1 and CCND1 upregulation and through other mechanisms^[21,22]. Other activated transcription factors implicated in tumor activity included RUNX transcription factor 1, melanocyte inducing transcription factor, and homeobox A10 (HOXA10)^[23-26]. Moreover, other top upstream regulators demonstrated varied biologic activity. For example, E1A binding protein P400 (EP400),

a component of the NuA4 histone acetyltransferase complex, is associated with epigenetic activity^[27]. Other upstream regulators are proteins involved in signaling pathways including actin like 6a, an actin binding protein involved in Notch1/SOX2 signaling, LIN9-MYBL2 [interacts with the tumor suppressors such as retinoblastoma (Rb) protein], SHC adaptor protein 1 (SHC1, a signaling adaptor for growth factor receptors), protein inhibitor of activated STAT 4 (PIAS4, a protein inhibitor of STAT), and Tumor necrosis factor (TNF) receptor associated factor 2 [TRAF2, role in TNF and nuclear factor-kappaB (NF-κB)]^[28-33]. We also found activation of RNA binding proteins including ELAVL1, a regulator of ferroptosis in hepatic stellate cells, as well as oncogenic members of the negative elongation factor family NELFE, NELFA, and NELFCD^[34]. Lastly, we found activation of oncogenesis-promoting kinases such as mitogen activated kinase 4 and neurotrophic receptor kinase 2 (NTRK2), which aid in cell adhesion^[35,36].

We next focused on inhibited upstream regulators (Supplementary Table 5), several of which are implicated in the innate immune response including the innate receptor DExD/H-Box helicase 58 (DDX58), or RIG-I, and the pattern recognition and toll-like receptors (TLR)2, TLR3, TLR4, and TLR9^[37,38]. There was predicted inhibition and downregulation of inflammatory mediators NF-κB and interleukins interleukin (IL)-5, IL-12, IL-18, and IL-33. We also found downregulation and predicted inhibition of the immune co-stimulatory molecule CD86, as well as forkhead head transcription factor FOXO3, a mediator of the antioxidant response and autophagy^[39]. Several other inhibited upstream regulators are well-described tumor suppressors such as TP53, CDKN1A, Rb gene, and Rb transcriptional suppressor type 2^[40]. Additionally, we found predicted inhibition of hepatocyte nuclear factors HNF4 and HNF4A, and the LXR NR1H3, which have been shown to exhibit anti-tumor activity^[41-44]. We also found downregulation and predicted inhibition of the transcription factor CCAAT enhancer binding protein delta (CEBPD), a regulator of apoptosis and potential tumor suppressor^[45]. Lastly, we observed downregulation and predicted inhibition of the SAM

domain, SH3 domain, and nuclear localization signals 1 (SAMS1), a lung cancer tumor suppressor that is hypermethylated in HCC^[46,47].

Causal networks of HBV-HCC

To elucidate the pathologic potential of the upstream regulators described above, we assessed downstream effector genes through IPA. We focused on upstream regulators with high activation z-scores. We first investigated RABL6 as it is the most activated upstream regulator in our analysis and its target genes play important roles in HBV-HCC (see Supplementary Table 4). Most of the activated genes are involved in promoting cell division. For example, the mitotic spindle checkpoint genes BUB1 and BUB1B, several cyclins including CCNA2, CCNB2, and CCNE2, and the M-phase inducer CDC25C were all upregulated^[48]. Likewise, we observed upregulation of centromere protein F, helicase RAD54B, and topoisomerase TOP2A. We also found upregulation of the mitogen PBK, NEK2 (a regulator of mitotic progression), and the kinase TTK, all of which promote HCC cell proliferation and migration *via* Akt signaling^[12,13,49]. Similarly, we found upregulation of minichromosome maintenance family (MCMs) members MCM2 and MCM10, which also promote cell division^[50-52]. There are also downstream genes of RABL6 with recently described roles in cancer. For example, we found upregulation of the ubiquitin-conjugating enzyme E2C. Knockdown of this gene has been shown to suppress cellular proliferation, migration, and invasion in HCC^[53]. Lastly, RABL6 may mediate HCC progression through downregulation of enhancer of zeste homolog 2, which regulates histone and DNA methylation and silences tumor suppressors^[54,55]. Thus, our analysis suggests RABL6 promotes HCC through several pro-oncogenic mechanisms. A summary of the downstream effects of RABL6 are presented in Figure 3.

Since RABL6 has not been described in HCC, we conducted survival analysis using the GEPIA2 platform. GEPIA2 is a website uses patient samples from TCGA used for bioinformatic analysis on genes of interest among different cancer types^[56]. We examined prognosis based on quartile expression of RABL6 (upper 75% *vs* lower 25%) in HCC

patients. We found a statistically lower chance of survival with higher expression of RABL6.

The upstream regulators TBX2, E2F1, FOXM1, and EP400 share similar activated genes to those described for RABL6 (see Supplementary material), such as HOXA10, and thus may act synergistically to promote HBV-HCC. This prompted us to next study activated genes downstream of HOXA10 due to its stark upregulation and high activation z-score (see Supplementary Table 6). While role of HOXA10 is not well-defined in HCC, knockdown model has been recently shown to inhibit HCC cell tumorigenesis^[26]. Additionally, another study by Shao *et al*^[57] demonstrated the involvement of HOXA10 in the renewal and survival of liver tumor initiation cells. IPA identified several genes downstream of HOXA10 that may explain its pathogenic activity. For example, HOXA10 may induce tumor progression through downregulation of the tumor suppressor gene NDRG2 as well as glutathione S-transferase A3 or GSTA3, whose inactivity results in hepatocyte oxidative stress and liver injury^[58,59]. We also found upregulation of dickkopf-1, a negative regulator of Wnt signaling and negative prognosticator for HCC^[60]. Insulin-like growth factor binding protein-3 is a potential mediator of growth suppression signals and is downregulated in our dataset^[61]. The hepatic enzyme CYP2E1 was similarly downregulated and is known to be repressed in HCC and linked with a poor prognosis^[62]. Lastly, xanthine dehydrogenase, a rate-limiting enzyme in purine metabolism, was downregulated and its suppression has been linked to enhanced cancer stem-cell activity in HCC^[63]. A suggested model of the potential multifactorial role HOXA10 and its interplay in HCC is shown in Figure 4.

Next, we investigated the downstream signaling of PIAS4 given its significant upregulation and activation z-score (see Supplementary Table 4). PIAS4 involvement in HCC has just been recently described^[32]. Downstream of PIAS4, we found upregulation of lymphoid enhancer factor 1 and downregulation of protocadherin 9, both of which promote epithelial-mesenchymal transition in HCC^[64-66]. We also found downregulation of fatty acid binding protein 1, which has been shown to reduce

oxidative stress, a major contributor to HCC development^[67]. Its loss may also lead to microsatellite instability in colorectal carcinomas and may have similar effects in HCC^[68]. Furthermore, we found downregulation of albumin, with evidence showing albumin itself suppressing HCC cellular proliferation^[69]. These results suggest a mechanistic role for PIAS4 in HCC progression (Figure 5).

Lastly, we investigated how inhibition of SAMS1 may contribute to HCC. As mentioned previously, SAMS1 inhibition is linked to malignant HCC tumorigenesis^[46]. From our IPA analysis, inhibition of SAMS1 has immunologic implications including downregulation of the pattern recognition receptor TLR3 and macrophage receptor with collagenous structure. These changes are known to negatively impact HCC prognosis^[37,70]. Furthermore, we found downregulation of the de-ubiquitinase USP12, which complexes with WD repeat protein WDR48 to suppress Akt signaling and tumor cell survival^[71]. Our suggested model of molecular mechanisms linking SAMS1 inhibition with HCC is showed in Figure 5.

DISCUSSION

Despite robust vaccination strategies in some countries, hepatitis B infection remains a leading global cause of liver cancer^[1,3]. Therapeutic options for HBV-related HCC remain poor owing to an overall lack of understanding of pathways involved in HBV oncogenesis. Current literature suggests HCC development is a result of aberrant activation of cellular signaling processes such as Wnt/FZD/ β -catenin, PI3K/Akt/mTOR, IRS1/IGF, and Ras/Raf/MAPK^[72]. Even with such knowledge, directed therapy for HBV-related HCC cases requires a more detailed understanding of the interactome. In this meta-analysis, we found potential underlying cellular pathways that define HBV-related HCC and its disease mechanisms. Our results build upon known contributors to HBV-related HCC including LXR/FXR/RXR signaling, Akt signaling, and immunological changes within the tumor microenvironment that are favorable for both HBV infection and tumor progression. We also illustrate the possible

activities of upstream regulators, whose role in HBV-related are not well described, such as RABL6, HOXA10, PIAS4, and SAMSN1.

We began our analysis by studying the top canonical pathways identified by IPA, which included LXR/RXR activation, LPS-IL-1 mediated RXR inhibition, acetone degradation, melatonin degradation, and FXR/RXR activation. LXR heterodimerizes with RXR and bind to LXR response elements, directly regulating gene expression^[73]. LXR primarily regulates expression of genes essential for lipid metabolism and is a known HCC tumor-suppressor^[74]. Similarly, the bile acid regulator FXR (NR1H4) suppresses hepatocarcinogenesis and was starkly downregulated in our analysis^[75]. In addition, inhibition of the acetone degradation pathway in HCC suggests alteration hepatic ketone metabolism, suggesting an increase in acetone levels may serve as a disease biomarker^[76]. Lastly, we saw predicted inhibition of the melatonin degradation pathway, which would presumably lead to a rise in melatonin. Melatonin has been demonstrated to inhibit HCC progression through let7i-3p-mediated RAF1 suppression^[77]. Overall, the top canonical pathways above reinforce the roles of LXR/RXR/FXR signaling in HCC pathogenesis and suggests a role for melatonin degradation in HBV-related HCC.

Top up- and downregulated genes identified in our analysis are implicated in oncogenic cellular signaling and other pathologic processes. For example, GPC3, the most upregulated gene in our analysis, functions as a co-receptor for Wnt proteins^[78]. Wnt signaling is vital for hepatobiliary function and cell differentiation. Therefore it's no surprise that aberrations in activity is a major contributor to HCC tumorigenesis and other liver disorders^[79]. GPC3 is also implicated in hedgehog signaling^[10], another important regulator of cell growth and differentiation; overactivation of which is associated with multiple cancer types including HCC^[80]. *In vitro* studies suggest GPC3 mediates cell proliferation through hedgehog signaling^[81]. Additionally, the negative hedgehog regulator HHIP was one of the most downregulated genes in our dataset^[16]. This change may act synergistically with GPC3 to further promote hedgehog signaling and cellular proliferation. Lastly, we found the kinase PBK as one of the most

upregulated genes. This kinase is related to the MAPKK family and has been recently been shown to promote HCC metastasis through ETV4a-uPar signaling^[12,82]. ETV4 is part of the ETS family of transcription factors and directly regulates cell division to promote pancreatic cancer and other cancer types^[83,84].

In addition to activation of cellular signaling pathways described above, our results also highlight changes in the tumor microenvironment and the immune response that may confer advantages to HBV infection and tumor progression. Our most downregulated genes included several members of the C-type lectin family including CLEC4G, CLEC1B, and CLEC4M. C-type lectins function in both the innate and adaptive immune response and downregulation of has been demonstrated in HCC^[14,85]. Lower expression of CLEC1B is associated with poorer outcomes in HCC and limits colon carcinoma metastasis^[86]. In addition, we found downregulation of pattern recognition receptors, like TLR3 and TLR2, implying impairment of the innate immune response. For example, TLR3, a receptor that recognizes viral components and double-stranded RNA^[87], has been associated with control of HBV infection and apoptosis of HCC cells^[37]. Likewise, TLR2, whose activity limits HCC cellular proliferation, was downregulated^[38]. We also noted downregulation of the innate immune receptor DDX58 or RIG-I. Elevated RIG-I expression limits HCC cellular proliferation and invasion^[88]. Moreover, HBV limits RIG-I signaling through induction of the miRNA miR146a^[89]. Lastly, other immunologic changes of note were repression of CD86 and IL-18. CD86 is a co-stimulatory molecule that has been well described as an anti-tumor response inducer through stimulation of cytotoxic T cells and other means^[90]. While the role of IL-18 in cancer is unclear, expression of IL-18 exhibits anti-tumor effects through the recruitment of tumor-infiltrating T cells^[91]. Thus, downregulation of IL-18 in the tumor microenvironment would be a prime contributor to HCC tumor progression.

Our analysis revealed FOXM1, E2F1, and EP400 as activated top upstream regulators in HBV-related HCC, each of which play a prominent role in facilitating cancer proliferation. FOXM1 has previously been shown to promote HCC progression *via* expression of genes KIF4A and CCNB1^[22]. Additionally, FOXM1 promotes tumor cell

proliferation *via* increasing expression of CCNB1 and CCND1, and decreasing expression of cell cycle checkpoint molecules p27 and p21^[92]. E2F1 is a transcription factor that has been shown to have both proliferative and apoptotic effects in HCC although the proliferative effects seem to be more prominent^[20]. E2F1 activates MYBL2 (another upregulated transcription factor in our analysis) and is involved in cell cycle progression^[93]. EP400 is a component of NuA4 histone acetyltransferase complex and is associated with activation of various genes. Recent studies have revealed it to be a critical transcription factor associated with greater HCC relapse and lower overall survival^[27].

In addition, our results showed RABL6, ESR1, NR0B2, and CEBPD as top upstream regulators that negatively regulate HCC tumor suppression. RABL6 is a member of Ras GTPase family that is overexpressed in HCC^[94]. Survival analysis suggests overexpression leads to a poorer prognosis (Figure 6). ESR1 was downregulated in our analysis, concordant with prior findings implicating ESR1 as a potential HCC tumor suppressor gene^[95]. Moreover, our results showed downregulation of NR0B2 and CEBPD. NR0B2 is a nuclear receptor and tumor suppressor; downregulation of which is associated with HCC and renal cell carcinoma^[96,97]. Similarly, CEBPD has also been posited as a candidate HCC tumor suppressor gene primarily through modulating IL-1 signaling^[98].

Interestingly, our results also revealed that HBV-related HCC progression may be intrinsically linked with repression of inflammatory and innate immune responses. Our analysis showed stark inhibition of the NF- κ B pathway, a Myc-dependent driver of HCC tumorigenesis^[99]. Indeed, other studies have proposed the NF- κ B pathway is an important mediator of hepatic fibrosis and disease progression, especially when inhibition is pronounced^[100]. While our meta-analysis allows for robust results of larger datasets, it has certain limitations. Annotations of public samples are limited and can introduce confounding variables to our analysis. For one, samples were taken from patients at different stages of HBV-related HCC. There may be significant differences in genetic aberrations based on tumor stage and grade. While samples were taken at the

time of diagnosis, patient characteristics like ethnicity, comorbidities, and medications were not clarified may still affect the results. Additionally, there may be differences in how samples were processed and how omics were performed between the studies included in our analysis. Of note, the studies in our meta-analysis did not clarify if changes in gene expression were attributable to HBV-DNA integration to the hepatocyte genome. Analysis focused on hepatocytes and the genes identified have link to HBV infection epiphonema as detailed above so a portion of the changes we have seen are linked to HBV. As explained, analysis focused on hepatocytes so gene changes would not be related to infiltrates. We did identify several genes involved in cell cycle regulation, but cellularity is important to function of these genes and could not be described in this approach. For future directions, we aim to validate our top genetic candidates using patient samples and control for such factors as stage of disease. We also hope to compare results between different etiologies of HCC such as HCV and alcohol. Doing so will elucidate the similarities and differences between these etiologies, allowing for a greater understanding of the oncogenic process while aiding the development of directed therapeutics for patient-specific treatment.

CONCLUSION

HBV is a leading cause of HCC and treatment options are still limited. In this meta-analysis based on public data, we studied the pathogenesis of HBV and pave the way for novel therapeutic avenues. We illustrated genetic changes that contribute to pro-oncogenic signaling through such pathways as the Akt, hedgehog, ETV4, and Wnt pathways. We also illustrated changes in the tumor microenvironment and immune response that are contributory to HBV infection and tumor progression. Additionally, we clarify the role of key upstream regulators such as RABL6, HOXA10, PIAS4, and SAMNS1 and describe how their downstream effects contribute to disease. These observations need to be further confirmed in prospective studies on oncogenesis. There is also need for investigating HBV-related cirrhosis and progressive changes of HBV-related HCC to assess the stepwise activity that define HBV oncogenesis.

ARTICLE HIGHLIGHTS

2

Research background

Hepatitis B virus (HBV) is a major cause of hepatocellular carcinoma (HCC) through several mechanisms including cirrhosis and direct oncogenic phenomena.

Research motivation

Studying HBV related HCC will offer novel insights to viral hepatic oncogenesis. It will also potentially lead to more directed therapy in HBV-related HCC.

Research objectives

Identify genetic changes and pathways that define HBV related HCC. Identify novel therapeutic targets.

Research methods

Used our novel Search Tag Analyze Resource platform to mine liver biopsies from HBV-related HCC patients and used ingenuity pathway analysis to study the results of our meta-analysis.

Research results

Our meta-analysis highlighted several genes and pathways with oncogenic potential. Of note, we describe two potential novel mediators of oncogenesis in rab-like protein 6 (RABL6) and homeobox A10 (HOXA10).

Research conclusions

This meta-analysis describes possible roles of RABL6 and HOXA10 in the pathogenesis of HBV-related HCC. RABL6 and HOXA10 represent potential therapeutic targets and warrant further investigation.

Research perspectives

The next steps to our research is to validate RABL6 and HOXA10 relevance in HBV-related HCC using clinical samples and establish its mechanistic underpinnings in an animal model.

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