From cirrhosis to hepatocellular carcinoma: An investigation into hepatitis C viral oncogenesis

Jihad Aljabban[1], Nilay Danis[2], Merve Gurakar[3], Kamal Khorfan[4], Nabeal Aljabban[5], Cem Simsek[2], Hussam Salhi[1], Maryam Panahiazar[6], Dexter Hadley[6], Ahmet Gurakar[2], Behnam Saberi[7]

[1]Ohio State University College of Medicine, Columbus, Ohio, USA; [2]Department of Medicine, Division of Gastroenterology and Hepatology, Johns Hopkins Medical Institutions, Baltimore, MD, USA; [3]Department of Medicine, Johns Hopkins Medical Institutions, Baltimore, MD, USA; [4]Henry Ford Health Systems, Detroit, Michigan, USA; [5]Penn State College of Medicine, Hershey, Pennsylvania 17033; [6]Institute for Computational Health Sciences, University of California, San Francisco, California 94158; [7]Icahn School of Medicine at Mount Sinai Division of Liver Diseases, New York, New York, USA

Abstract

Background and Aim: Hepatitis C is a leading cause of chronic liver disease and hepatocellular carcinoma (HCC). Understanding the evolution and biology of HCC among HCV patients may lead to novel therapeutic avenues and risk stratification.

Material and Methods: Using meta-analysis platform STARGEO, we performed two separate meta-analyses as follows: 357 HCV-related HCC tumor samples with 220 adjacent non-tumor samples and 92 HCV-related cirrhotic liver samples with 53 healthy liver samples as a control. Then, we analyzed the signature in Ingenuity Pathway Analysis.

Results: HCV cirrhosis analysis demonstrated LPS/IL-1 mediated inhibition of RXR function, LXR/RXR activation, sirtuin signaling, IL-10 signaling and hepatic fibrosis/stellate cell activation as top canonical pathways. IL1β, TNF, and TGF-β1 were top upstream regulators. Cellular morphologic and signaling changes were noted through the up-regulation of RGS1/2, WNT receptor FZD7, the TGF-β1-induced gap junction gene GJA1, and the zinc finger transcription factor repressor SNAI2. Apoptosis was inhibited through the down-regulation of OMA1. Metabolic dysfunction was noted through the down-regulation of SCLY and CBS. HCV-related HCC analysis showed FXR/RXR and LXR/RXR signaling, LPS/IL1-mediated inhibition of RXR activation, and melatonin degradation as top canonical pathways.

Conclusion: Our results suggest that the genetic changes in the setting of chronic HCV infection predispose patients to developing HCC.

Keywords: HCC; HCV; STARGEO.

Introduction

Hepatitis C virus (HCV) is a leading cause of liver disease with chronic infection, potentially leading to cirrhosis in approximately 20–30% of the infective patients.[1] HCV patients with cirrhosis are at hepatocellular carcinoma (HCC) development with an annual rate of ≈ 3.5%.[2] In

direct-acting antivirals (DAAs), era sustained virologic response (SVR) rates exceed 95% in HCV infection.[3] However, the opioid epidemic has led to a rise in the incidence of HCV across the globe.[4,5] In the era of DAAs, we are able to cure the majority of the HCV patients. HCC risk persists, especially in patients with advanced cirrhosis. First reports about the occurrence or recurrence of HCC after achieving SVR with DDAs were conflicting; some articles alleged potentially increased risk of HCC occurrence or recurrence,[6,7] sequential reports refuted this argument.[8,9] Moreover, new reports showed treatment with DAAs improved survival of HCV infected, even cirrhotic patients.[3,10–12] Despite advancements in treatment in HCC, prognosis remains poor. Predicting which cirrhotic HCV patients will develop HCC remains a challenge. In the DAA cured patients, we still do not have long term data given that the INF free drugs only available since 2014. Factors that influence HCC *de-novo* occurrence or recurrence are being widely investigated. Male sex, diabetes, liver stiffness measurement and fibrosis-4 score were found independently associated with *de-novo* HCC, whereas diabetes was the only independent risk factor for recurrent HCC.[13] Another study found out a lack of SVR and alpha-fetoprotein (AFP) as predictors of recurrent HCC.[14] Understanding the evolution of liver fibrosis to HCC in HCV will pave the way for improved risk stratification and the development of novel therapeutic avenues. Nowadays, genes alterations in HCC following DAA treatment and pathological pathways from cirrhosis to HCC in HCV are being investigated.[15] In this study, we aimed to characterize better the pathways involved in the oncogenesis of chronic HCV infection, and demonstrate the utility of crowd-sourced data and our STARGEO platform in the investigation of HCV-related HCC.[16]

Materials and Methods

The National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO) is an open database of millions of biological samples from functional genomics experiments. The Search Tag Analyze Resource for GEO (STARGEO) platform allows for meta-analysis of genomic signatures of disease and tissue through tagging of biological samples from several experiments. More information on STARGEO and its functionality can be found in our previous paper.[16] To study the different stages of progression from cirrhosis to HCC in HCV patients, we conducted a separate meta-analysis. For the meta-analysis of the cirrhotic stage of the disease, we tagged 92 HCV-related cirrhotic liver samples and tagged 53 healthy liver samples as a control from
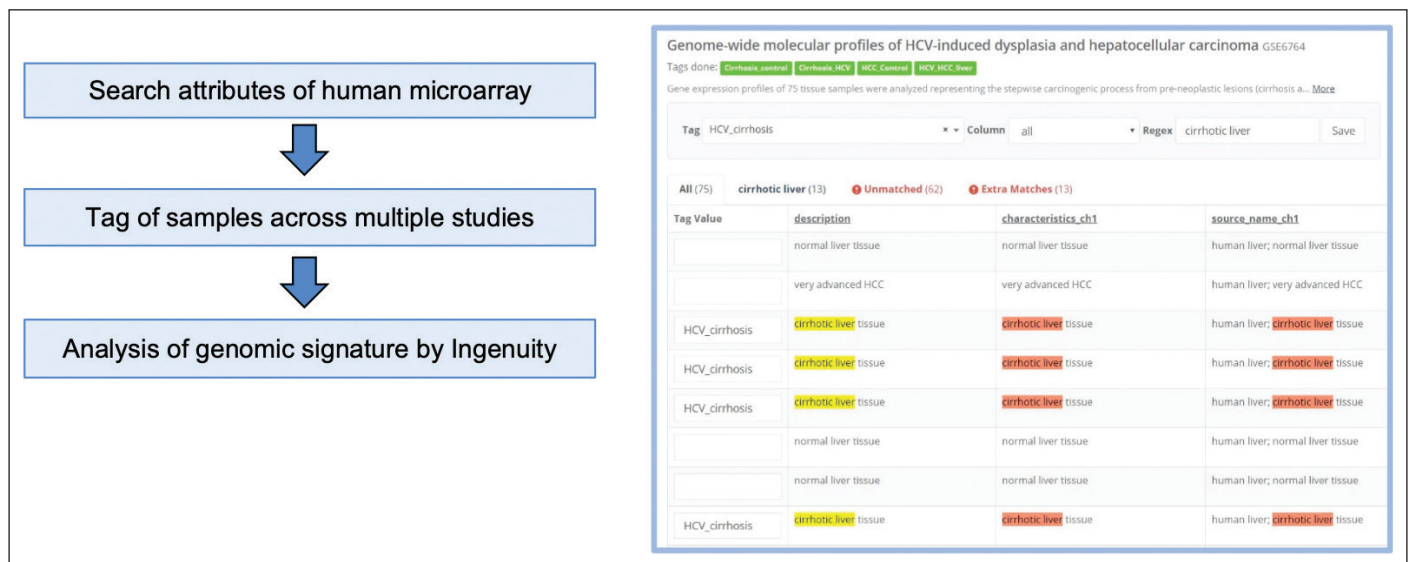
Figure 1. A screen capture of how to use STARGEO. Relevant experiments can be searched and samples from those experiments can be tagged using the regex function, as shown.

STARGEO: Search tag analyze resource for GEO; GEO: Gene expression omnibus.

Table 1. Summary of the list genes that are the most up-regulated and down-regulated in our meta-analysis of HCV-related cirrhosis and HCC. Experimental log ratios are shown

Top up-regulated				Top down-regulated			
HCV-Cirrhosis		HCV-HCC		HCV-Cirrhosis		HCV-HCC	
RGS1	0.834	DUXAP10	1.185	OMA1	-0.527	LINC01093	-2.258
PTGER4	0.581	NMRAL2P	1.130	SAA2	-0.313	CNDP1	-2.062
FZD7	0.552	CRNDE	1.125	LRIF1	-0.252	CLEC4G	-1.928
RGCC	0.524	AKRB10	1.090	CCDC69	-0.228	OIT3	-1.816
GJA1	0.416	CTHRC1	1.073	WWP1	-0.227	CLRN3	-1.814
DAB2	0.385	GPC3	1.072	SCLY	-0.211	TTC36	-1.763

a total of three series. For the meta-analysis of the HCC stage of the disease, we tagged 357 HCV-related HCC liver tumor samples and 220 adjacent non-tumor samples as a control from a total of 8 series (Fig. 1). Patients from these studies were not treated with DAAs. We were able to extract 1000s of genes for each of the meta-analyses conducted used STARGEO (see Table 1 for top up- and down-regulated genes). To evaluate this data, we analyzed the gene signatures from our meta-analyses in Ingenuity Pathway Analysis (IPA), restricting genes that showed statistical significance ($p<0.05$) and an absolute experimental log ratio greater than 0.15 between conditions and control samples. These selected genes have been used for the next step analysis in IPA to elucidate the biological process, mechanisms of disease, and potential biomarkers and therapeutic targets that will be highlighted in our results and discussion section in this study.

IPA is based on the QIAGEN knowledge base and highlights biological pathways, drugs, and disease processes for OMICs data based on the most up-to-date literature. IPA contains millions of facts on the relationship between genes, disease processes, phenotype, drug activity, and more that can be searched for and highlighted in inputted genetic studies. These facts come from genomic experiments from several modal-

ities, including SNP and micro-RNA microarrays, RNA-sequencing, proteomic and metabolomic studies, chemical lists, and more. IPA allows us to dissect the complex biological networks that characterize genomic, metabolomic, and proteomic data.[17] IPA allows us to take advantage of the novelty of our approach in using large scale data, and results from IPA analysis are demonstrated below.

All data analyzed were taken from Gene Expression Omnibus. There was no interaction or intervention with human subjects and no involvement with access with identifiable private patient information. As such, no IRB approval was necessary.

Results

HCV-Related Cirrhosis Analysis

We start with our analysis of HCV-related cirrhotic liver tissue. IPA analysis from our HCV-related cirrhosis study demonstrated LPS/IL-1 mediated inhibition of RXR (retinoid X receptor) function (p-value 4.38 E-06; z-score -1.633), LXR (liver X receptor)/RXR activation (p-value 5.10 E-06; z-score 0.707), sirtuin signaling (p-value 6.09 E-05; z-score -1.265), and IL-10 signaling (p-value 8.33 E-05; z-score NaN)
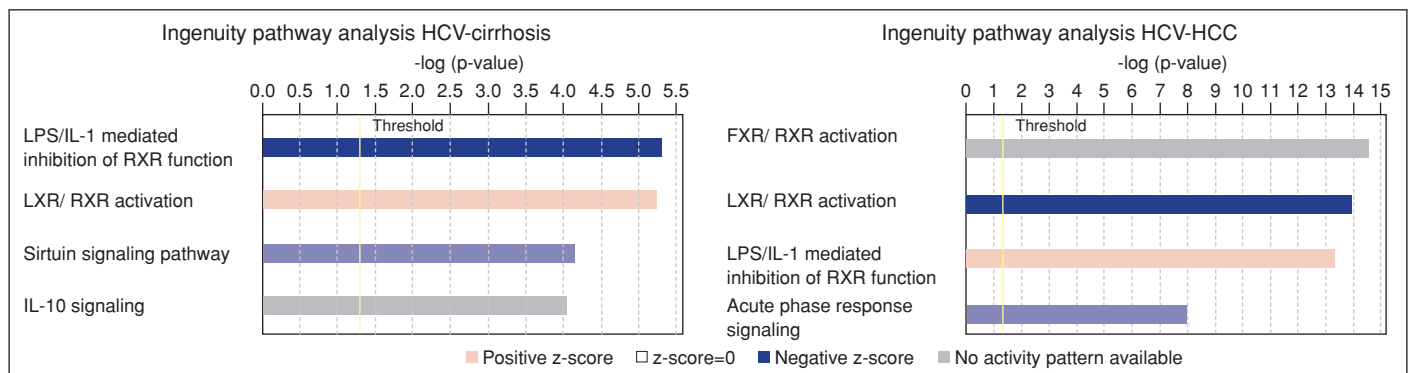
Figure 2. Top canonical pathways identified by Ingenuity Pathway Analysis from the HCV-related cirrhosis meta-analysis (above) and the HCV-related HCC meta-analysis. Z-score is illustrated in the legend.

(Fig. 2). From our HCV-related cirrhosis IPA analysis, we also identified interleukin 1-beta or IL1-β (p-value 8.03 E-11), transforming growth factor-beta or TGF-β (with predicted activation; p-value 3.49 E-76), tumor necrosis factor or TNF (with predicted activation; p-value 7.49 E-73), and interferon-gamma or IFN-γ (with predicted activation; p-value 2.33 E-49) as top upstream regulators. Our meta-analysis showed significant up and down-regulation of 1000s of genes between cirrhotic samples and healthy controls. The top up- and down-regulated genes have potential involvement in HCV infectivity, and oncogenesis is summarized in Table 1.

Among our most up-regulated genes were prostaglandin E2 receptor 4 or PTGER4 (p-value 0.0476, experimental log ratio 0.581). We also noted cell signaling pathways linked to oncogeneses, such as the up-regulation of genes involved in G protein-coupled signaling RGS1 (p-value 0.0412, experimental log ratio 0.834) and RGS2 (p-value 0.00, experimental log ratio 0.282), and in the pro-oncogenic pathway aryl hydrocarbon signaling, such as TIPARP (p-value 0.00, experimental log ratio 0.171). We found the up-regulation of the frizzled gene receptor FZD7. Additionally, we found up-regulation of RGCC, or regulator of cell cycle and known as RGC-32, (p-value 0.0188, experimental log ratio 0.524). We also noted up-regulation of genes gap junction alpha protein 1 or GJA1 (p-value 0.0172, experimental log ratio 0.416) and SNAI2 (p-value 0.00, experimental log ratio 0.321). We found the down-regulation of pyridoxal kinase (PDKX).

HCV-Related Hepatocellular Carcinoma Analysis

IPA analysis from our HCV-related HCC analysis demonstrated FXR (farnesoid X receptor)/RXR (p-value 1.41 E-10; z-score NaN) and LXR/RXR signaling (p-value 2.03 E-10; z-score -2.043), and LPS/IL1-mediated inhibition of RXR activation (p-value 2.12 E-12; z-score 1.633) as top canonical pathways. Our meta-analysis demonstrated the down-regulation of NR0B2 (p-value 4.51 E-4, experimental log ratio -0.204). From our HCV-related HCC IPA analysis, we also identified ERBB2 (with predicted activation, p-value 2.26 E-26), PPARA (p-value 7.16 E-26), ZBTB17 (p-value 2.77 E-23), CDKN1A (with predicted inhibition, p-value 1.08 E-21), and calcitriol (with predicted inhibition, p-value 1.24 E-21). We next investigated the relationship between calcitriol activity as an upstream regulator and genes relevant to tumor control or progression using. IPA predicted that the decrease in calcitriol activity leads to downstream down-regulation of transcription factors and tumor suppressors CEPBD (p-value 1.27 E-4, experimental log ratio -0.190) and EGR1 (p-value 5.51 E-5, experimental log ratio

-0.446). We also noted a down-regulation of interferon regulatory factor 8 (IRF8; p-value 0.00216, experimental log ratio -0.232). The pro-apoptotic factor FAS (p-value 0.0196, experimental log ratio -0.151) was also down-regulated. Additionally, we found the down-regulation of the retinoid receptor RXRa (p-value 4.62 E-7, experimental log ratio -0.284). Lastly, we found up-regulation of pro-oncogenic transcription factor FOXM1[18–20] (p-value 3.38 E-6, experimental log ratio 0.371) and oncogenic receptor tyrosine kinase KIT (p-value 0.0209, experimental log ratio 0.165).

The top two most up-regulated genes were the recently described oncogenic pseudogenes DUXAP10 (p-value 0.00312, experimental log ratio 1.18) and NMRAL2P (p-value 1.99 E-4, experimental log ratio 1.13). Additionally, we found up-regulation of the long non-coding RNA CRNDE (colorectal neoplasia differentially expression; p-value 4.41 E-4, experimental log ratio 0.320). We also found up-regulation of the gene collagen triple helix repeating containing 1 or CTHRC1 (p-value 4.05 E-5, experimental log ratio 0.261). Next, we wanted to investigate up-regulation of canonical beta-catenin/TCF targets given their role in cancer and found up-regulation of AXIN2 (p-value 3.59 E-4, experimental log ratio 0.383), LEF1 (p-value 1.98 E-8, experimental log ratio 0.677), and DKK1 (p-value 0.00345, experimental log ratio 0.230).

Discussion

HCV-Related Cirrhosis Analysis

IPA analysis from our HCV-related cirrhosis study demonstrated LPS/IL-1 mediated inhibition of RXR. The top canonical pathways highlighted reveal an overall activation of the LXR and RXR pathways at this stage of the disease. RXR and LXR are responsible for regulating lipid metabolism[21] and may play a role in anti-HCV activity as HCV replication is linked to increased lipid metabolism.[22] HCV core protein binds to RXRα and induces the activity of lipid metabolism enzymes, further supporting the role of lipid metabolism in HCV pathogenesis. Transcriptomic analysis of *in vitro* HCV replication has implicated activation of the "LPS/IL-1 mediated inhibition of RXR function" and inhibition of the "PXR/RXR" activation pathways in HCV activity.[23] Given the role of lipid metabolism in HCV replication, this activity pattern may serve as an anti-viral response that is lost in the chronic infection phase of HCV, as would be seen in the cirrhotic liver samples we studied. Another potential role for these pathways is their potential role in cancer. Retinoids have a strong connection with HCC, with loss of retinoid activity being seen in HCC cell lines.[22] Altered retinoid signaling and decreased stores of retinoid have been noted in both cir-
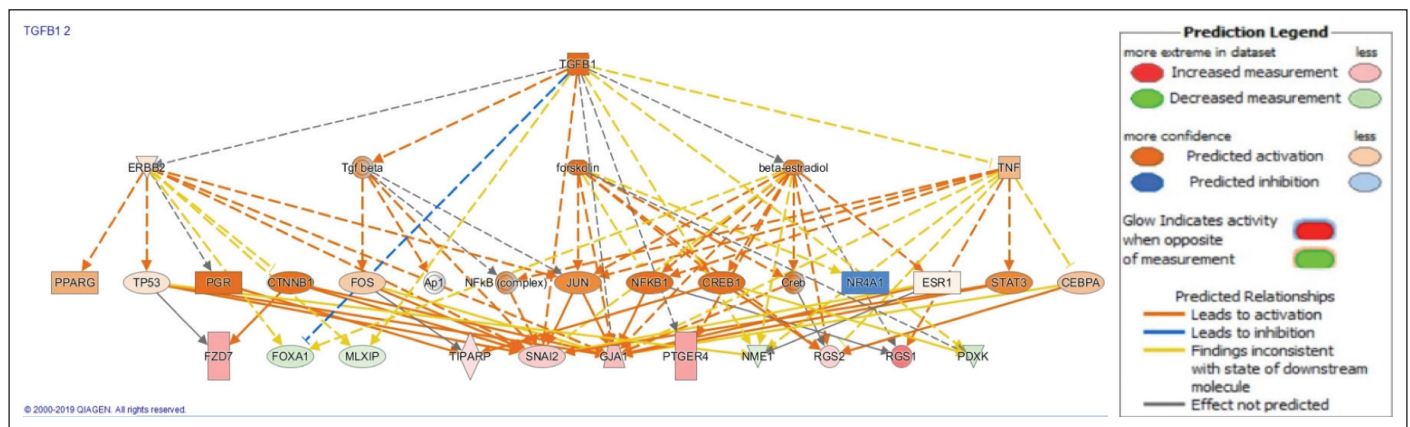
Figure 3. Ingenuity pathway analysis of several candidate genes with oncogenic and tumor suppressing properties downstream of TFGB1. Prediction legend below shows relations of genes.

rhosis and HCC.[23] These past studies have motivated efforts in using acyclic retinoid in HCC prevention and treatment of liver diseases.[24] Our results illustrate the overall agonism of RXR activity and may represent an anti-oncogenic response at the cirrhotic stage of the disease. RXR agonism may decrease over the course of the cirrhotic disease to the point where there is antagonism in RXR signaling at the point of HCC, as discussed below.

IL1-β, TGF-β, TNF, IFN-γ genes are top upstream regulators in HCV-related cirrhosis according to our analysis. All these genes have well-established roles in inflammation and are actively studied in the context of cirrhosis and liver disease. For example, HCV infection has been shown to induce IL1-β secretion in exposed Kupffer cells (resident liver macrophages) and, subsequently, drive hepatic inflammation.[25] TGF-β has been shown to partake in all stages of disease progression from fibrosis to cirrhosis, and finally, to HCC.[26] TGF-β contributes to liver fibrosis is through activation of hepatic stellate cells, differentiation of stellate cells to activated myofibroblasts, and apoptosis of hepatocytes.[26] Although TGF-β has tumor-suppressive activity in the early stages of tumorigenesis, strong activation of TGF-β signaling is linked to tumor progression and may serve as a therapeutic target for HCC.[27,28] The role of TNF in liver fibrosis and cirrhosis is not fully understood, but recent studies demonstrate its pivotal role.[29] TNF signaling leads to hepatic stellate activation and survival and modulates other immune cells, including macrophages, dendritic cells, and B cells in contributing to liver fibrosis and cirrhosis.[30,31] While the relationship between IFN-γ and stages of the disease have not been fully elucidated, there is a strong association between elevations of IFN-γ throughout all these stages.[32] Additionally, IFN-γ has been shown to exacerbate liver damage in mouse models.[33]

Our meta-analysis showed significant up-and down-regulation of 1000s of genes between cirrhotic samples and healthy controls. The top up- and down-regulated genes have potential involvement in HCV infectivity and oncogenesis (Table 1). We wanted to focus on our results and discussion on genes involved in liver fibrosis and progression of cirrhosis to HCC. One of our most up-regulated genes was prostaglandin E2 receptor 4 or PTGER4. Prostaglandin E2 has been shown to synergistically impair cytotoxic T cell function leading to the development of chronic infections, such as in HCV,[34] and promotion of cancer, including obesity-related HCC.[35] We also noted cell signaling pathways linked to oncogenesis, such as up-regulation of genes involved in G protein-coupled signaling RGS1, RGS2, and in the pro-oncogenic pathway aryl hydrocarbon signaling, such as TIPARP.[36–39] We found the up-

regulation of the frizzled gene receptor FZD7, which causes increased Wnt/Beta-catenin activity and is associated with HCC.[40] Soluble FZD7 inhibits Wnt signaling and sensitizes HCC cell lines towards doxorubicin.[41] Additionally, we found up-regulation of RGCC, or regulator of cell cycle and known as RGC-32, which is involved in cell proliferation, fibrosis, and cancer.[42–46] Deficiency of RGC-32 can be protective from hepatic fibrosis, and potentially HCC, by limiting lipogenesis.[47] We also noted the up-regulation of genes involved in cell adhesion and progression to malignancy, including gap junction alpha protein 1 or GJA1 and SNAI2. GJAI has been associated with HCC[48] and SNAI2 is a zinc finger transcriptional repressor which induces down-regulation of E-cadherin and promotes progression to HCC.[49] Lastly, we found down-regulation of tumor-suppressive genes, including FOXA1 (p-value 0.00, experimental log ratio -0.143), OMAI (p-value 0.0298 E-7, experimental log ratio -0.527), NME1 (p-value 2.22 E-16, experimental log ratio -0.110 and PDXK (p-value 0.00, experimental log ratio -0.163). NMEI and FOXA1 have recently been shown to suppress HCC tumor cell viability through suppressing PI3K/Akt signaling.[50,51] OMAI is a mitochondrial metalloprotease activated by Bax and Bak, leading to apoptosis and, its dysregulation has been linked to HCC.[52] We found the down-regulation of PDKX required for vitamin B6 synthesis. Vitamin B6 has potent antioxidative effects and its deficiency may have a causative role in HCC.[53] Since TGF-β is a top upstream regulator in our analysis and is implicated in liver disease progression, we illustrated its downstream effects on the genes discussed above (Fig. 3).

HCV-Related Hepatocellular Carcinoma Analysis

A fraction of patients with HCV-related cirrhosis will develop HCC. We conducted two separate meta-analyses as described above to illustrate the genetic drivers of this disease progression. The LXR/RXR activation pathway and LPS/IL-1 mediated inhibition of RXR activation are discussed above. As opposed to our HCV-related cirrhosis analysis, in this analysis, we see the overall activation of the RXR pathway. As discussed above, RXR agonism has an oncogenic effect and would be expected at this stage of the disease. FXR is a key regulator of bile acid synthesis and homeostasis.[54,55] FXR also regulates the enterohepatic circulation of bile acids.[56] Proper regulation of bile acids is paramount as it is toxic in excess, and improper FXR activity may cause the progression of inflammatory bowel disease, gallstones disease, liver fibrosis, and HCC.[57–59] FXR activity may limit hepatic inflammation and, by extension, progression of liver disease. While IPA identified FXR sig-
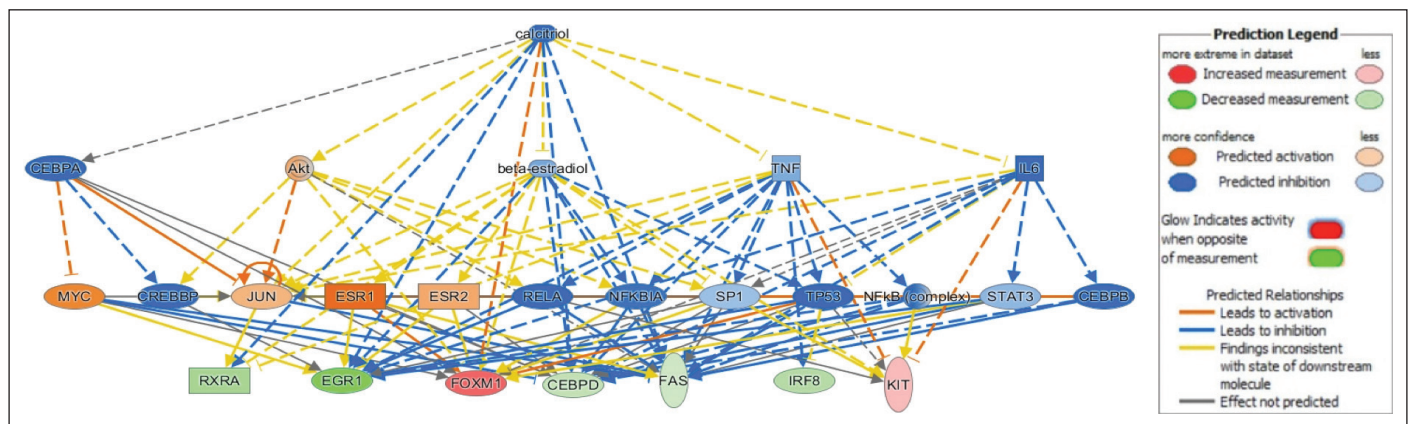
Figure 4. Ingenuity pathway analysis of several candidate genes with oncogenic and tumor suppressing properties downstream of calcitriol. Prediction legend below shows relations of genes.

naling as a top canonical pathway, it did not predict whether this pathway was activated or inhibited. We investigated the expression pattern of its target genes NR0B2, Abcb11, Ibabp, Osta, and Ostb. Our meta-analysis demonstrated down-regulation of NR0B2, but we did not have information on the other genes described, which may suggest inhibition of FXR signaling and subsequent promotion of tumor progression.

From our HCV-related HCC IPA analysis, we also identified ERBB2, PPARA, ZBTB17, CDKN1A, and calcitriol. ERBB2 has an underappreciated role in the context of HCC, but recent evidence links ERBB2 expression to HCC stage and tumor recurrence.[60] PPARA, or peroxisome proliferator-activated receptor alpha, is a major regulator of lipid metabolism with evidence for anti-tumor activity and a potential role for regulation of HCC progression.[61] ZBTB17 is a zinc finger protein that maintains MYC expression, a known contributor to HCC and hepatoblastoma.[62] Using MYC activity, ZBTB17 may sustain tumor maintenance. CDKN1A, or p21, is a target of p53 and is linked to cell arrest in response to DNA damage.[63] CDKN1A may inhibit HCC tumorigenesis.[64] Our analysis predicts inhibition of its activity and, presumably, inhibition of cell arrest. Lastly, calcitriol has been shown to regulate the cell cycle, promote cell differentiation, and demonstrate anti-tumor activity within the tumor micro-environment.[65] Calcitriol is being investigated as a potential therapy in HCC.[66,67] We illustrated how the downstream effects of calcitriol inhibition on tumor suppressor and oncogene expression (Fig. 4). Downstream of calcitriol, we found down-regulation of transcription factors and tumor suppressors CEBPD[68] and EGR1.[69] We also noted IRF8, which functions as a tumor suppressor in some solid tumors.[70,71] The pro-apoptotic factor FAS was also down-regulated. Additionally, we found down-regulation of the retinoid receptor RXRa, which would promote tumor progression as above. Lastly, we found the up-regulation of pro-oncogenic transcription factor FOXM1[72–74] and oncogenic receptor tyrosine kinase KIT.

Next, we want to highlight several of the top up-regulated oncogenic genes. The top two most up-regulated genes were the recently described oncogenic pseudogenes DUXAP10 and NMRAL2P. DUXAP10 has been shown to promote the progression of non-small cell lung cancer (NSCLC) through interaction with oncogenic proteins and repression of the tumor-suppressive proteins.[72] NMRAL2P was identified as a transcriptional target of the transcription factor Nrf2,[73] which promotes the development of tumors.[74] The silencing of NMRAL2P through CRISPR/Cas leads to inhibition of cancer cell growth and migration.[73] While not previously studied in HCC, DUXAP10 and NMRAL2P may

have similar activity as described above. Additionally, we found up-regulation of the long non-coding RNA CRNDE (colorectal neoplasia differentially expression). CRNDE promotes cell survival, migration, and cancer cell proliferation in several cancer types.[75] We also found the up-regulation of the gene CTHRC1. CTHRC1 enhances the adhesion and migratory activity of cancer cells and is linked with poor prognosis in HCC patients.[76] When we investigated the up-regulation of canonical beta-catenin/TCF targets given their role in cancer and found up-regulation of AXIN2, LEF1, and DKK1. These target genes were not up-regulated in our HCV-related cirrhosis analysis and suggested a difference in beta-catenin activity as the disease progresses. Beta-catenin is expressed in the cell junction of hepatocytes and regulates cellular adhesion and communication.[77] Alterations of this pathway are common in the development of HCC.[77]

Conclusion

Our investigation illustrated the genetic changes in the setting of chronic HCV infection and cirrhosis that predispose patients to developing HCC. Some of these changes, such as LXR/FXR signaling and anti-tumor immune response, persist from the cirrhotic to the carcinoma stage. The other changes characterize what pathways and genes may drive progression from cirrhosis to HCC and may serve as potential therapeutic targets and biomarkers from liver biopsy analysis for patients at high risk for developing HCC. In the future, we plan on expanding our data set to investigate the immune micro-environment and to validate our results with patient samples.

References

1. Seeff LB. Natural history of chronic hepatitis C. Hepatology 2002;36:S35-S46.

2. Sangiovanni A, Del Ninno E, Fasani P, De Fazio C, Ronchi G, Romeo R, et al. Increased Survival of Cirrhotic Patients with a Hepatocellular Carcinoma Detected during Surveillance. Gastroenterology 2004;126(4):1005-1014.

3. Cabibbo G, Celsa C, Calvaruso V, Petta S, Cacciola I, Cannavò MR, et al; Rete Sicilia Selezione Terapia – HCV (RESIST-HCV) and Italian Liver Cancer (ITA.LI.CA.) Group. Direct acting antivirals after successful treatment of early hepatocellular carcinoma improve survival in HCV-cirrhotic patients. J Hepatol 2019;71(2):265-273.

4. Morris MD, Mirzazadeh A, Evans JL, Briceno A, Coffin P, Hahn JA, et al. Treatment cascade for hepatitis C virus in young adult people who inject drugs in San Francisco: Low number treated. Drug Alcohol Depend 2019;198:133-135.

5. Cooper CL, Galanakis C, Donelle J, Kwong J, Boyd R, Boucher L, et al. HCV-infected individuals have higher prevalence of comorbidity and multimorbidity: a retrospective cohort study. BMC Infectious Diseases 2019;19(1):712.

6. Conti F, Buonfiglioli F, Scuteri A, Crespi C, Bolondi L, Caraceni P, et al. Early occurrence and recurrence of hepatocellular carcinoma in HCV-related cirrhosis treated with direct-acting antivirals. J Hepatol 2016;65(4):727-733.

7. Reig M, Mariño Z, Perelló C, Iñarrairaegui M, Ribeiro A, Lens S, et al. Unexpected high rate of early tumor recurrence in patients with HCV-related HCC undergoing interferon-free therapy. J Hepatol 2016;65(4):719-726.

8. Kanwal F, Kramer J, Asch SM, Chayanupatkul M, Cao Y, El-Serag HB. Risk of Hepatocellular Cancer in HCV Patients Treated With Direct-Acting Antiviral Agents. Gastroenterology 2017;153(4):996-1005.e1.

9. Ioannou GN, Green PK, Berry K. HCV eradication induced by direct-acting antiviral agents reduces the risk of hepatocellular carcinoma. J. Hepatol 2017.

10. Dang H, Yeo YH, Yasuda S, Huang CF, Iio E, Landis C, et al. Cure with Interferon Free DAA is Associated with Increased Survival in Patients with HCV related HCC from both East and West. Hepatology 2019.

11. Fehily SR, Papaluca T, Thompson AJ. Long-Term Impact of Direct-Acting Antiviral Agent Therapy in HCV Cirrhosis: Critical Review. Semin Liver Dis 2019;39(3):341-353.

12. Colussi G, Donnini D, Brizzi RF, Maier S, Valenti L, Catena C, et al. Sustained virologic response to direct-acting antiviral agents predicts better outcomes in hepatitis C virus-infected patients: A retrospective study. World J Gastroenterol 2019;25(40):6094-6106.

13. Degasperi E, D'Ambrosio R, Iavarone M, Sangiovanni A, Aghemo A, Soffredini R, et al. Factors Associated With Increased Risk of De Novo or Recurrent Hepatocellular Carcinoma in Patients With Cirrhosis Treated With Direct-Acting Antivirals for HCV Infection. Clin Gastroenterol Hepatol 2019;17(6):1183-1191.e7.

14. Lleo A, Aglitti A, Aghemo A, Maisonneuve P, Bruno S, Persico M, et al. Predictors of hepatocellular carcinoma in HCV cirrhotic patients treated with direct acting antivirals. Dig Liver Dis 2019;51(2):310-317.

15. Sghaier I, Brochot E, Loueslati BY, Almawi WY. Hepatitis C virus protein interaction network for HCV clearance and association of DAA to HCC occurrence via data mining approach: A systematic review and critical analysis. Reviews in Medical Virology 2019;29:e2033.

16. Hadley D, Pan J, El-Sayed O, Aljabban J, Aljabban I, Azad TD, et al. Precision annotation of digital samples in NCBI's gene expression omnibus. Sci Data 2017;4:170125.

17. Krämer A, Green J, Pollard J Jr, Tugendreich S. Causal analysis approaches in Ingenuity Pathway Analysis. Bioinformatics 2014;30(4):523-530.

18. Nakamura S, Hirano I, Okinaka K, Takemura T, Yokota D, Ono T, et al. The FOXM1 transcriptional factor promotes the proliferation of leukemia cells through modulation of cell cycle progression in acute myeloid leukemia.

Carcinogenesis 2010;31(11):2012-2021.

19. Park HJ1, Carr JR, Wang Z, Nogueira V, Hay N, Tyner AL, et al. FoxM1, a critical regulator of oxidative stress during oncogenesis. EMBO J 2009;28(19):2908-2918.

20. Chen X, Müller GA, Quaas M, Fischer M, Han N, Stutchbury B, et al. The forkhead transcription factor FOXM1 controls cell cycle-dependent gene expression through an atypical chromatin binding mechanism. Mol Cell Biol 2013;33(2):227-236.

21. Zimmerman TL, Thevananther S, Ghose R, Burns AR, Karpen SJ. Nuclear export of retinoid X receptor alpha in response to interleukin-1-beta-mediated cell signaling: Roles for JNK and SER260. J Biol Chem 2006;281(22):15434-15440.

22. Matsushima-Nishiwaki R, Okuno M, Takano Y, Kojima S, Friedman SL, Moriwaki H. Molecular mechanism for growth suppression of human hepatocellular carcinoma cells by acyclic retinoid. Carcinogenesis;24(8):1353-1359.

23. Shirakami Y, Lee SA, Clugston RD, Blaner WS. Hepatic metabolism of retinoids and disease associations. Biochim Biophys Acta 2012;1821(1):124-136.

24. Shimizu M, Imai K, Takai K, Moriwaki H. Role of Acyclic Retinoid in the Chemoprevention of Hepatocellular Carcinoma: Basic Aspects, Clinical Applications, and Future Prospects. Curr Cancer Drug Targets 2012;12(9):1119-1128.

25. Shrivastava S, Mukherjee A, Ray R, Ray RB. Hepatitis C Virus Induces Interleukin-1 (IL-1)/IL-18 in Circulatory and Resident Liver Macrophages. J Virol 2013;87(22):12284-12290.

26. Fabregat I, Moreno-Càceres J, Sánchez A, Dooley S, Dewidar B, Giannelli G, et al. TGF-β signalling and liver disease. FEBS J 2016;283(12):2219-2232.

27. Giannelli G, Villa E, Lahn M. Transforming growth factor-β as a therapeutic target in hepatocellular carcinoma. Cancer Res 2014;74(7):1890-1894.

28. Neuzillet C, de Gramont A, Tijeras-Raballand A, de Mestier L, Cros J, Faivre S, et al. Perspectives of TGF-ß inhibition in pancreatic and hepatocellular carcinomas. Oncotarget 2014;5(1):78-94.

29. Yang YM, Seki E. TNFα in Liver Fibrosis. Curr Pathobiol Rep 2015;3(4):253-261.

30. Szabo G, Csak T. Inflammasomes in liver diseases. J Hepatol 2012;57(3):642-654.

31. Li T, Ma H, Chiang JY. TGFβ1, TNFα, and insulin signaling crosstalk in regulation of the rat cholesterol 7α-hydroxylase gene expression. J Lipid Res 2008;49(9):1981-1989.

32. Attallah AM, El-Far M, Zahran F, Shiha GE, Farid K, Omran MM, et al. Interferon-gamma is associated with hepatic dysfunction in fibrosis, cirrhosis, and hepatocellular carcinoma. J Immunoassay Immunochem 2016;37(6):597-610.

33. Knight B, Lim R, Yeoh GC, Olynyk JK. Interferon-γ exacerbates liver damage, the hepatic progenitor cell response and fibrosis in a mouse model of chronic liver injury. J Hepatol 2007;47(6):826-833.

34. Chen JH, Perry CJ, Tsui YC, Staron MM, Parish IA, Dominguez CX, et al. Prostaglandin E2 and programmed cell death 1 signaling coordinately impair CTL function and survival during chronic viral infection. Nat Med 2015;21(4):327-334.

35. Loo TM, Kamachi F, Watanabe Y, Yoshimoto S, Kanda H, Arai Y, et al. Gut microbiota promotes obesity-associated liver cancer through pge2-mediated suppression of antitumor immunity. Cancer Discov 2017;7(5):522-538.

36. Talari NK, Panigrahi MK, Madigubba S, Phanithi PB. Overexpression of aryl hydrocarbon receptor (AHR) signalling pathway in human meningioma. J Neurooncol 2018;137(2):241-248.

37. Grimaldi G, Rajendra S, Matthews J. The aryl hydrocarbon receptor regulates the expression of TIPARP and its cis long non-coding RNA, TIPARP-AS1. Biochem Biophys Res Commun 2018;495(3):2356-2362.

38. MacPherson L, Tamblyn L, Rajendra S, Bralha F, McPherson JP, Matthews

J. 2,3,7,8-Tetrachlorodibenzo-p-dioxin poly(ADP-ribose) polymerase (TiPARP, ARTD14) is a mono-ADP-ribosyltransferase and repressor of aryl hydrocarbon receptor transactivation. Nucleic Acids Res 2013;41(3):1604-1621.

39. Cheng L, Li Z, Huang YZ, Zhang X, Dai XY, Shi L, et al. TCDD-inducible poly-ADP-ribose polymerase (TIPARP), a novel therapeutic target of breast cancer. Cancer Manag Res 2019;11:8991-9004.

40. Merle P, de la Monte S, Kim M, Herrmann M, Tanaka S, Von Dem Bussche A, et al. Functional consequences of frizzled-7 receptor overexpression in human hepatocellular carcinoma. Gastroenterology 2004;127(4):1110-1122.

41. Wei W, Chua MS, Grepper S, So SK. Soluble Frizzled-7 receptor inhibits Wnt signaling and sensitizes hepatocellular carcinoma cells towards doxorubicin. Mol Cancer 2011;10:16.

42. Vlaicu SI, Cudrici C, Ito T, Fosbrink M, Tegla CA, Rus V, et al. Role of response gene to complement 32 in diseases. Arch Immunol Ther Exp (Warsz) 2008;56(2):115-122.

43. Li F, Luo Z, Huang W, Lu Q, Wilcox CS, Jose PA, et al. Response gene to complement 32, a novel regulator for transforming growth factor-β-induced smooth muscle differentiation of neural crest cells. J Biol Chem 2007;282(14):10133-10137.

44. Li Z, Xie WB, Escano CS, Asico LD, Xie Q, Jose PA, et al. Response gene to complement 32 is essential for fibroblast activation in renal fibrosis. J Biol Chem 2011;286(48):41323-41330.

45. Fosbrink M, Cudrici C, Niculescu F, Badea TC, David S, Shamsuddin A, et al. Overexpression of RGC-32 in colon cancer and other tumors. Exp Mol Pathol 2005;78(2):116-122.

46. Kim DS, Lee JY, Lee SM, Choi JE, Cho S, Park JY. Promoter methylation of the RGC32 gene in nonsmall cell lung cancer. Cancer 2011;117(3):590-596.

47. Cui XB, Luan JN, Chen SY. RGC-32 deficiency protects against hepatic steatosis by reducing lipogenesis. J Biol Chem 2015;290(33):20387-20395.

48. Cleary SP, Jeck WR, Zhao X, Chen K, Selitsky SR, Savich GL, et al. Identification of driver genes in hepatocellular carcinoma by exome sequencing. Hepatology 2013;58(5):1693-1702.

49. Zhao X, Sun B, Sun D, Liu T, Che N, Gu Q, et al. Slug promotes hepatocellular cancer cell progression by increasing sox2 and nanog expression. Oncol Rep 2015;33(1):149-156.

50. He S, Zhang J, Zhang W, Chen F, Luo R. FOXA1 inhibits hepatocellular carcinoma progression by suppressing PIK3R1 expression in male patients. J Exp Clin Cancer Res 2017;36(1):175.

51. Khan I, Gril B, Steeg PS. Metastasis suppressors NME1 and NME2 promote dynamin 2 oligomerization and regulate tumor cell endocytosis, motility, and metastasis. Cancer Res 2019;79(18):4689-4702.

52. Dai W, Jiang L. Dysregulated Mitochondrial Dynamics and Metabolism in Obesity, Diabetes, and Cancer. Front Endocrinol (Lausanne) 2019;10:570.

53. Cheng SB, Lin PT, Liu HT, Peng YS, Huang SC, Huang YC. Vitamin B-6 supplementation could mediate antioxidant capacity by reducing plasma homocysteine concentration in patients with hepatocellular carcinoma after tumor resection. Biomed Res Int 2016;2016:7658981.

54. Makishima M, Okamoto AY, Repa JJ, Tu H, Learned RM, Luk A, et al. Identification of a nuclear receptor for bite acids. Science 2019;284:1362-1365.

55. Chiang JY. Bile acids: Regulation of synthesis. J Lipid Res 2009;50(10):1955-1966.

56. Lu TT, Makishima M, Repa JJ, Schoonjans K, Kerr TA, Auwerx J, et al. Molecular basis for feedback regulation of bile acid synthesis by nuclear receptos. Mol Cell 2000;6(3):507-515.

57. Lu Y, Ma Z, Zhang Z, Xiong X, Wang X, Zhang H, et al. Yin Yang 1 promotes hepatic steatosis through repression of farnesoid X receptor in obese mice. Gut 2014;63(1):170-178.

58. Su H, Ma C, Liu J, Li N, Gao M, Huang A, et al. Downregulation of nuclear receptor FXR is associated with multiple malignant clinicopathological

59. Gadaleta RM, van Erpecum KJ, Oldenburg B, Willemsen EC, Renooij W, Murzilli S, et al. Farnesoid X receptor activation inhibits inflammation and preserves the intestinal barrier in inflammatory bowel disease. Gut 2011;60(4):463-472.

60. Shi JH, Guo WZ, Jin Y, Zhang HP, Pang C, Li J, et al. Recognition of HER2 expression in hepatocellular carcinoma and its significance in postoperative tumor recurrence. Cancer Med 2019;8(3):1269-1278.

61. Hsu HT, Chi CW. Emerging role of the peroxisome proliferator-activated receptor-gamma in hepatocellular carcinoma. J Hepatocell Carcinoma 2014;1:127-135.

62. Kress TR, Pellanda P, Pellegrinet L, Bianchi V, Nicoli P, Doni M, et al. Identification of MYC-dependent transcriptional programs in oncogene-addicted liver tumors. Cancer Res 2016;76(12):3463-3472.

63. Waldman T, Kinzler KW, Vogelstein B. p21 Is Necessary for the p53-mediated G1 Arrest in Human Cancer Cells. Cancer Res 1995;55(22):5187-5190.

64. Zeng Y, Shen Z, Gu W, Wu M. Inhibition of hepatocellular carcinoma tumorigenesis by curcumin may be associated with CDKN1A and CTGF. Gene 2018;651:183-193.

65. Díaz L, Díaz-Muñoz M, García-Gaytán AC, Méndez I. Mechanistic effects of calcitriol in cancer biology. Nutrients 2015;7(6):5020-5050.

66. Rizvi A, Farhan M, Naseem I, Hadi SM. Calcitriol–copper interaction leads to non enzymatic, reactive oxygen species mediated DNA breakage and modulation of cellular redox scavengers in hepatocellular carcinoma. Apoptosis 2016;21(9):997-1007.

67. Rizvi A, Rizvi G, Naseem I. Calcitriol induced redox imbalance and DNA breakage in cells sharing a common metabolic feature of malignancies: Interaction with cellular copper (II) ions leads to the production of reactive oxygen species. Tumour Biol 2015;36(5):3661-3668.

68. Pan YC, Li CF, Ko CY, Pan MH, Chen PJ, Tseng JT, et al. CEBPD reverses RB/E2F1-mediated gene repression and participates in HMDB-induced apoptosis of cancer cells. Clin Cancer Res 2010;16(23):5770-5780.

69. Inoue K, Fry EA. Tumor suppression by the EGR1, DMP1, ARF, p53, and PTEN Network. Cancer Invest 2018:1-17.

70. Zhang Q, Zhang L, Li L, Wang Z, Ying J, Fan Y, et al. Interferon regulatory factor 8 functions as a tumor suppressor in renal cell carcinoma and its promoter methylation is associated with patient poor prognosis. Cancer Lett 2014;354(2):227-234.

71. Ye L, Xiang T, Zhu J, Li D, Shao Q, Peng W, et al. Interferon consensus sequence-binding protein 8, a tumor suppressor, suppresses tumor growth and invasion of non-small cell lung cancer by interacting with the wnt/β-Catenin pathway. Cell Physiol Biochem 2018;51(2):961-978.

72. Wei CC, Nie FQ, Jiang LL, Chen QN, Chen ZY, Chen X, et al. The pseudogene DUXAP10 promotes an aggressive phenotype through binding with LSD1 and repressing LATS2 and RRAD in non small cell lung cancer. Oncotarget 2017;8(3):5233-5246.

73. Johnson GS, Li J, Beaver LM, Dashwood WM, Sun D, Rajendran P, et al. A functional pseudogene, NMRAL2P, is regulated by Nrf2 and serves as a coactivator of NQO1 in sulforaphane-treated colon cancer cells. Mol Nutr Food Res 2017;61(4).

74. DeNicola GM1, Karreth FA, Humpton TJ, Gopinathan A, Wei C, Frese K, et al. Oncogene-induced Nrf2 transcription promotes ROS detoxification and tumorigenesis. Nature 2011;475(7354):106-109.

75. Zhang J, Yin M, Peng G, Zhao Y. CRNDE: An important oncogenic long non-coding RNA in human cancers. Cell Prolif 2018;51:e12440.

76. Chen YL, Wang TH, Hsu HC, Yuan RH, Jeng YM. Overexpression of CTHRC1 in Hepatocellular Carcinoma Promotes Tumor Invasion and Predicts Poor Prognosis. PLoS One 2013;8(7):e70324.

77. Khalaf AM, Fuentes D, Morshid AI, Burke MR, Kaseb AO, Hassan M, et al. Role of Wnt/β-catenin signaling in hepatocellular carcinoma, pathogenesis, and clinical significance. J Hepatocell Carcinoma 2018;5:61-73.