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

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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. IL-1β, 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/IL-1-mediated inhibition of RXR activation, and melanotin 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.[10,11,12] 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...
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 modalities, 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).
Hepatocellular Carcinoma Analysis

IPA analysis from our HCV-related cirrhosis study demonstrated the up-regulation of growth factor-beta (p-value 0.0476, experimental log ratio 0.581). We also noted a positive canonical pathway linked to oncogenes, 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). Additionally, we found up-regulation of the frizzled gene receptor FZD7. 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 a negative canonical pathway linked to tumor progression, such as the down-regulation of genes including FOXM1 and KIT (p-value 0.0209, experimental log ratio -0.284). Lastly, we found up-regulation of pro-oncogenic transcription factor FOXM1 (p-value 0.38 E-6, experimental log ratio 0.371) and oncogenic receptor tyrosine kinase KIT (p-value 0.0209, experimental log ratio 0.165).

Discussion

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The top two most up-regulated genes were the recently described oncogenic pseudogenes DUXAP10 (p-value 0.03 E-4, experimental log ratio 0.18) and NMRAL2P (p-value 0.19 E-4, experimental log ratio 0.13). Additionally, we found up-regulation of the long non-coding RNA CRNDE (colorectal neoplasia differentially expressed; p-value 0.41 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 0.05 E-5, experimental log ratio 0.261). The pro-oncogenic pathway is further supported by our findings of the up-regulation of the long non-coding RNA CRNDE (colorectal neoplasia differentially expressed; p-value 0.41 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 0.05 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 0.39 E-4, experimental log ratio 0.383), LEF1 (p-value 0.98 E-8, experimental log ratio 0.677), and DKK1 (p-value 0.00345, experimental log ratio 0.230).

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We also noted cell signaling pathways.

Additionally, we found up-regulation of RGCC, or regulator of FXR activity may limit hepatic inflammation and, by limitation, implicated in liver disease progression, we illustrated its downstream effects on the genes discussed above (Fig. 3).

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.

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. FXR also regulates the enterohpatic circulation of bile acids. 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, gallstone disease, liver fibrosis, and HCC. FXR activity may limit hepatic inflammation and, by extension, progression of liver disease. While IPA identified FXR sig-

regulation of the frizzled gene receptor FZD7, which causes increased Wnt/Beta-catenin activity and is associated with HCC. Soluble FZD7 inhibits Wnt signaling and sensitizes HCC cell lines towards doxorubicin. 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. Deficiency of RGC-32 can be protective from hepatic fibrosis, and potentially HCC, by limiting lipogenesis. We also noted the up-regulation of genes involved in cell adhesion and progression of liver disease. While IPA identified FXR signaling, such as TIPARP, 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. We found the up-regulation of several candidate genes with oncogenic and tumor suppressing properties downstream of TFGB1. Prediction legend below shows relations of genes.
We also noted IRF8, which functions as a tumor suppressor and oncogene expression (Fig. 4). Downstream of calcitriol, we found down-regulation of transcription factors and tumor suppressors CEBPD and EGR1. We also noted IRF8, which functions as a tumor suppressor in some solid tumors. 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 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.[52] NMRAL2P was identified as a transcriptional target of the transcription factor Nr12,[53] which promotes the development of tumors.[54] The silencing of NMRAL2P through CRISPR/Cas leads to inhibition of cancer cell growth and migration.[55] 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 expressed). CRNDE promotes cell survival, migration, and cancer cell proliferation in several cancer types.[56] 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.[57] 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.[58] Alterations of this pathway are common in the development of HCC.[59]

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.

Ethics Committee Approval: 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.

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