Sex-Differential Expression Analysis in Viral-Mediated Hepatocellular Carcinoma.

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ABSTRACT

Hepatocellular Carcinoma (HCC) is the second deadliest cancer worldwide and is increasing in prevalence in most countries. The majority of HCC cases worldwide are virally-mediated with around 50% mediated by hepatitis B virus (HBV) and 25% mediated by hepatitis C virus (HCV). HCC also exhibits sex-differences with significantly higher incidence and worse prognosis in males. The mechanistic basis of these sex-differences is poorly understood. To identify genes and pathways that are sex-differentially expressed in viral-mediated HCC, we performed differential expression analysis on tumor vs. tumor adjacent samples that were stratified based on sex and viral etiology. We calculated the log fold change (logFC) for all genes between female tumor and tumor-adjacent tissue or male tumor and tumor-adjacent tissue. We then fit a linear regression to the log fold change of each gene between males and females. Top sex-differentially expressed candidates were identified as those with the greatest difference from the linear model. Genes showing the greatest difference between males and females included MUC13, and MAGEA6 for HBV-mediated liver cancer and CDHR2, IGF2, and H19 for HCV-mediated liver cancer. Each of these genes has a previous association with HCC including known roles in the formation of HCC (IGF2) associations with poor prognosis (MUC13, MAGEA6), and prior evidence of tumor to tumor-adjacent differential expression (CDHR2, H19,). Understanding genes and pathways with different expression levels in male and females may contribute to improved individualized therapeutic or diagnostic options for HCC.

METHODS

- RNAseq data from tumor and tumor-adjacent hepatocellular carcinoma samples were obtained from the International Cancer Genome Consortium (ICGC) (Table 1).
- Differential expression analysis was performed using limma/voom. The results were stratified by both sex and viral etiology (Figure 1).
- Linear regression model fit for the tumor:tumor-adjacent log fold changes (logFC) in males vs. females to identify genes with a different expression pattern in males and females (Figure 2).
- Expression of top gene candidates from the linear regression analysis were represented visually using violin plots (Figure 3).
- A literature search was done on top gene candidates to identify previous associations with HCC and cancer overall (Table 2),

	Male Tumor	Male Adj.	Female Tumor	Female Adj
HBV	33	40	8	9
HCV	59	71	34	36

Table 1: Patient sample data taken from the ICGC. *More samples available for males than females.*

HBV VOLCANO PLOTS

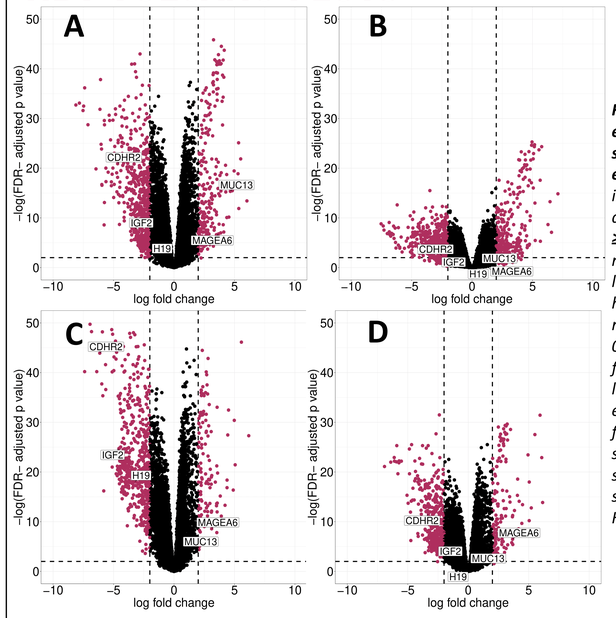

Figure 1: Differentially expressed genes stratified by sex and etiology,. *Maroon dots indicate genes with an absolute value of logFC ≥ 2. Vertical dashed lines represent an absolute logFC ≥ 2 and the horizontal dashed line represents a p-value of 0.05. Genes selected for further research are labeled. Differentially expressed genes shown for (A) male HBV samples (B) female HBV samples, (C) Male HCV samples, and (D) Male HCV samples.*

SEX SPECIFIC DIFFERENTIALLY EXPRESSED GENES

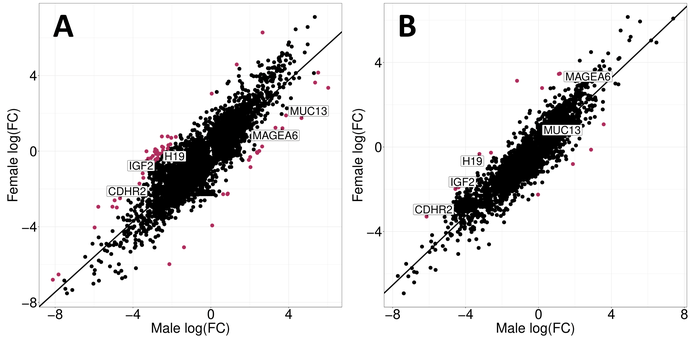

Figure 2: Linear Regression identifies genes with the greatest difference in the tumor:tumor-adjacent logFC between males and females. *Maroon dots indicate a residual ≥ 2.25. Genes selected for further research are labeled on the plot. Linear regression model for (A) HBV samples and (B) HCV samples.*

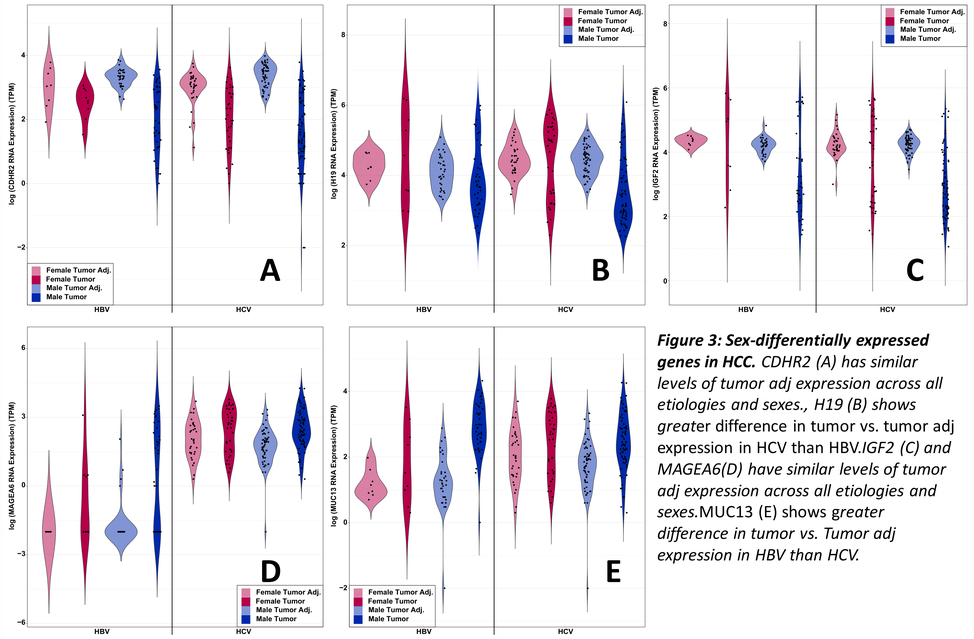

Figure 3: Sex-differentially expressed genes in HCC. *CDHR2 (A) has similar levels of tumor adj expression across all etiologies and sexes., H19 (B) shows greater difference in tumor vs. tumor adj expression in HCV than HBV.IGF2 (C) and MAGEA6(D) have similar levels of tumor adj expression across all etiologies and sexes.*MUC13 (E) shows *greater difference in tumor vs. Tumor adj expression in HBV than HCV.*

LITERATURE SEARCH

Gene	logFC Tumor vs. adj.	Reported differential expression	Reported Function
MUC13	Positive	• Previously shown to be higher in HCC than adjacent tissue (Dai, 2018)	• Overexpression associated with poor survival among patients with intrahepatic cholangiocarcinoma (Tiemin, 2020) • Associated with resistance to cancer cell death (Sheng, 2016) • Potential therapeutic target for colorectal cancers (Sheng, 2016)
MAGEA6	Positive		• Gene overexpression inhibits cell migration & invasion of HCC cells (Guo, 2019) • Downregulation of gene inhibits stemness maintenance of self-renewal of HCC stem cells (Guo, 2019)
H19	Negative		• Repressed in most tissues and re-expressed in many cancers (Gamaev, 2021) • Shown to act as an oncogene in mouse models (Gamaev, 2021)
CDHR2	Negative	• Previously shown to be higher in adjacent tissue than HCC (Xia, 2019)	• Downregulated in HCC cell lines and tissues (Xia, 2019) • Novel tumor suppressor in HCC growth in vitro and in vivo (Xia, 2019)
IGF2	Negative	• Previously shown to be overexpressed in HCC than adjacent tissues (Martinex-Quetglas	• Known to be overexpressed in HCCs (Martinez-Quetglas, 2016) • Accelerates formation of liver tumors through IGF 1 receptor signaling (Martinez-Quetglas, 2016)

Table 2 : Literature Search *shows reported expression and function of the five selected genes*

CONCLUSIONS

- CDHR2, IGF2, & MAGEA6 have similar levels of tumor adj expression across all etiologies and sexes
- H19 shows a greater difference in tumor tumor-adj expression in HCV than HBV
- MUC13 shows a greater difference in tumor tumor-adj expression in HBV than HCV

FUTURE WORK

- Pathway analysis to investigate potential relationships between genes of interest.
- Investigate additional genes with great difference between tumor tumor-adj logFC
- Differential Expression analysis with T2T-CHM13 reference genome

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