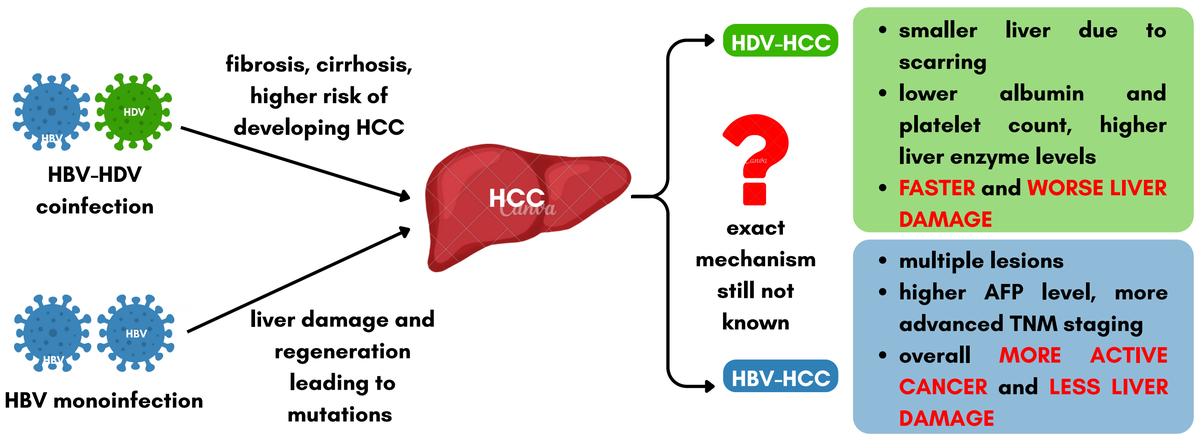
Integrated Bioinformatics Analysis of DEGs Reveals Specific Molecular Pathways Driving Poorer Prognosis in HDV-HCC Compared to HBV-HCC

Chloe Alexandra Sinatra[1], Nicholas Putra Lesmana[1], Stephen Dario Syofyan[1]

1. *Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia*

Introduction

Hepatocellular carcinoma (HCC) is the **5th most common cancer** worldwide and is the **2nd leading cause of death** in men. **HDV** is a **defective RNA virus** which requires HBV surface antigens (HBsAg) for viral assembly, and therefore presents as **HBV-HDV coinfection**. **HBV-HDV coinfection** is considered to be the **most aggressive form of chronic viral hepatitis**, having **4 times higher risk** of developing into **HCC**. **HBV associated HCC** often presents with **more lesions** and **more aggressive** tumor behavior, while **HDV associated HCC** has been shown to have **poorer prognosis results in worse liver damage.** However, the exact mechanisms behind this difference is **still unknown.**



Objective

This study aims to **identify key genes** and **molecular pathways** contributing to poorer **HDV-HCC prognosis** as compared to **HBV-HCC,** and to assess their potential **as therapeutic drug targets.**

Method

1. Gene Expression Omnibus was used to find datasets regarding HBV associated HCC (**GSE55092**) and HDV associated HCC (**GSE98383**).

2. The **GEO2R** tool was then used to identify DEGs between **healthy samples** and **HBV-HCC** and **HDV-HCC samples.**

3. **Microsoft Excel** was used to identify significant results using p adj < 0,05, and logFC > 1 and < -1 to identify **upregulated** and **downregulated** genes in both datasets

4. **Interactivenn** was used to compare upregulated and downregulated genes between **HBV-HCC and HDV-HCC,** in order to **identify differences** in **gene expression.**

5. **KEGG** and **Gene Ontology** analysis was conducted on the common DEGs using **Enrichr** Software

6. **Cytoscape** and the **CytoHubba plugin** was used to construct a **PPI-network** and identify top **Hub Genes**

Search Keywords *hbv-hcc"geo2r"[filter], hdv-hcc "geo2r"[filter]*

Results and Graphics

Study	Dataset	Samples (n)		Tissue	Protocol design
		HDV-HCC	No HCC		
Diaz G et. al 2018	GSE98383	5	7	Liver Tissue	Affymetrix Human Genome U133 Plus 2.0 Array
		Malignant	Non-Malignant		
Melis M et. al 2014	GSE55092	11	11	Liver Tissue	Affymetrix Human Genome U133 Plus 2.0 Array

Table 1. Characteristics of Included Studies

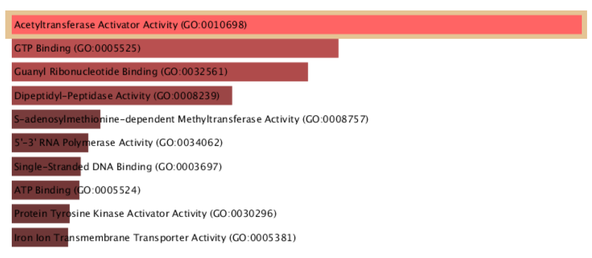


Figure 1. Molecular function of downregulated genes in HDV

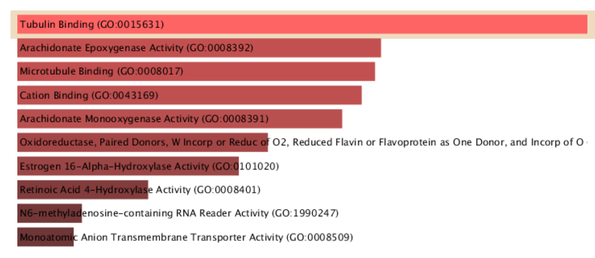


Figure 2. Molecular function of downregulated genes in HBV

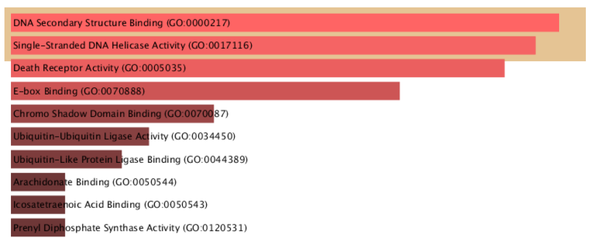


Figure 3. Molecular function of upregulated genes in HDV

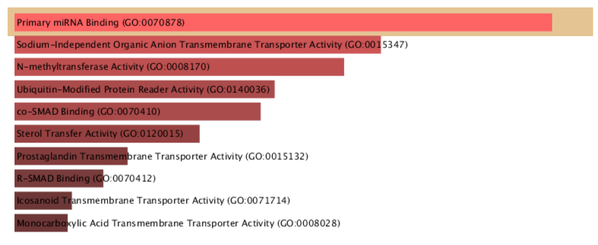


Figure 4. Molecular function of upregulated genes in HBV

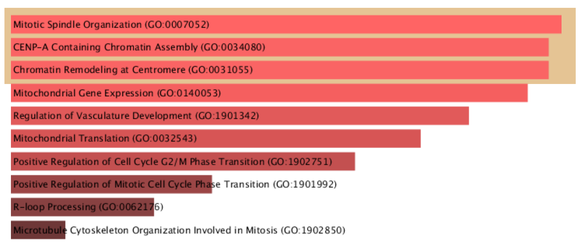


Figure 5. Biological process of downregulated genes in HDV

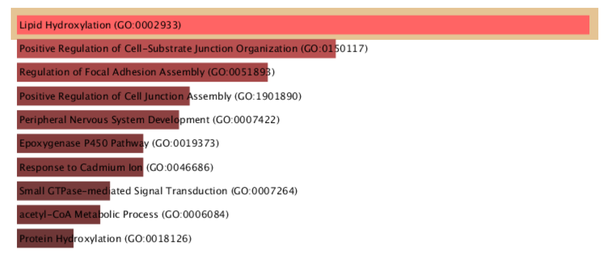


Figure 6. Biological process of downregulated genes in HBV

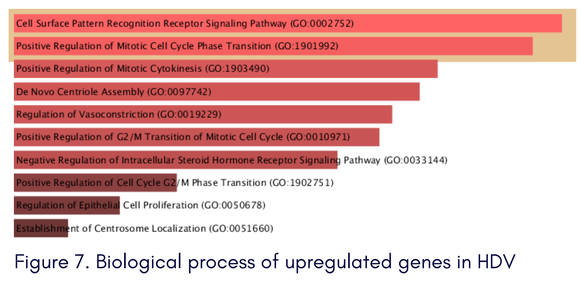


Figure 7. Biological process of upregulated genes in HDV

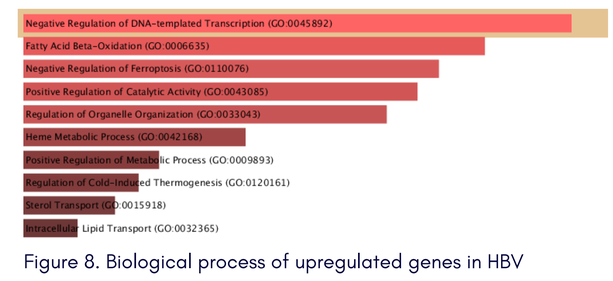


Figure 8. Biological process of upregulated genes in HBV

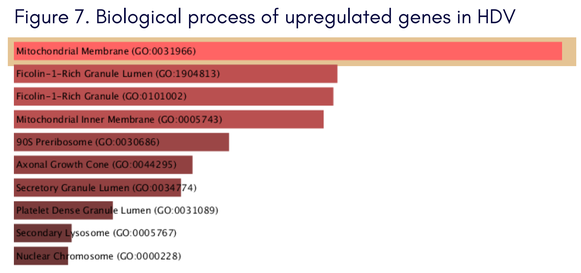


Figure 9. Cellular component of downregulated genes in HDV

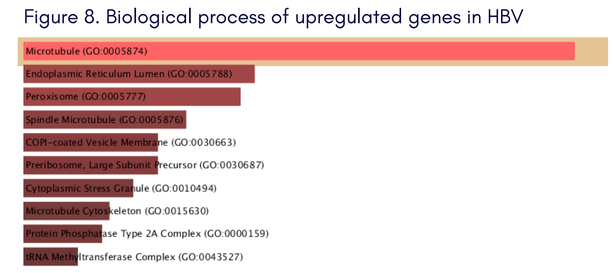


Figure 10. Cellular component of downregulated genes in HBV-HCC

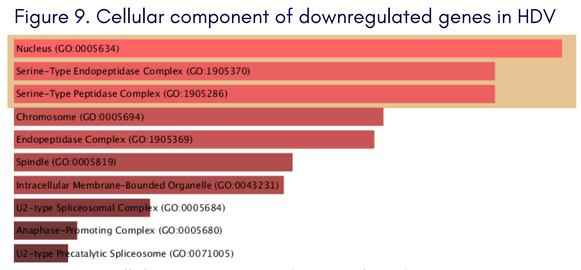


Figure 11. Cellular component of upregulated genes in HDV-HCC

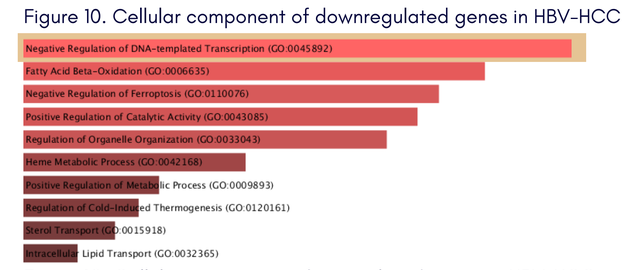


Figure 12. Cellular component of upregulated genes in HBV-HCC

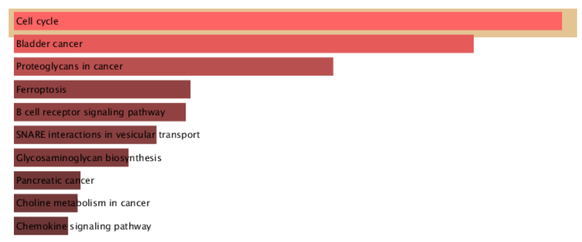


Figure 13. KEGG analysis of downregulated genes in HDV-HCC

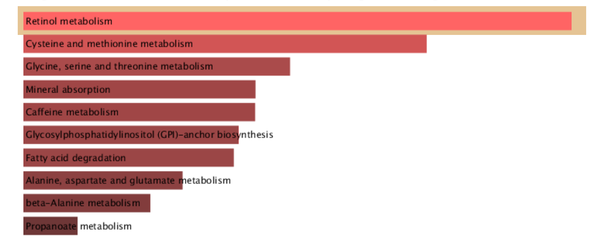


Figure 14. KEGG analysis of downregulated genes in HBV-HCC

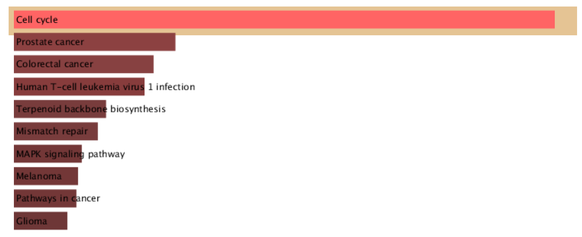


Figure 15. KEGG analysis of upregulated genes in HDV-HCC

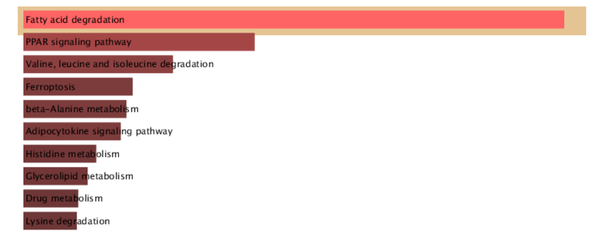


Figure 16. KEGG analysis of upregulated genes in HBV-HCC

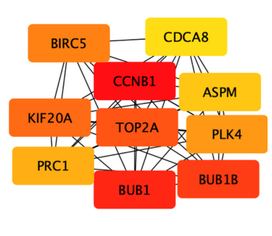


Figure 13. Upregulated hub genes in HDV-HCC

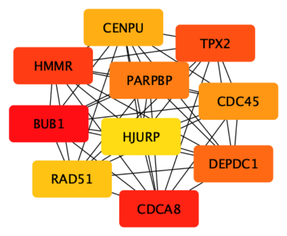


Figure 14. Downregulated hub genes in HDV-HCC

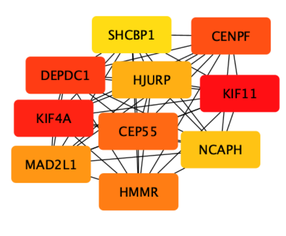


Figure 15. Upregulated hub genes in HBV-HCC

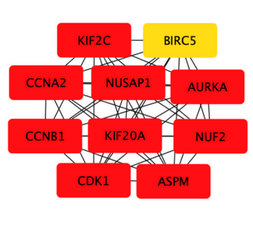


Figure 16. Downregulated hub genes in HBV-HCC

Discussion

HDV-HCC

UPREG: Cell surface pattern recognition receptor signalling pathway

- **Pattern Recognition Receptors (PRRs)** induces **pro-inflammatory cytokine production → inflammation, hepatocyte injury, regeneration = fibrosis**
- **HDV DYSREGULATES PRR signalling = oxidative stress and DNA damage**
- **chronic PRR activation = inflammation driven hepatocarcinogenesis**

DOWNREG: Mitotic Spindle Organization

- **HDV** disrupts **cell cycle regulation,** promotes **centrosome amplification, increase ROS** = more **DNA damage/errors in mitosis**
- **Possible biomarkers**
  - **AURKA → overexpression in HCC = poorer prognosis**
  - **PLK1 →** spindle checkpoint control **inhibited = increased HCC growth**
  - **BUB1/BUBR1 → mutated in HCC = premature completion of mitosis**

HBV-HCC

UPREG: Negative regulation of DNA-templated transcription

- **HBV interfere with transcriptional machinery → tumor supressor genes are SUPPRESSED → oncogenes are activated**
- **HBx → activate oncogenic pathways → suppresses tumor supressors**
- **HBsAg → induces ER (endoplasmic reticulum stress) AND IL-6 signalling → repress anti-tumor immune response**

DOWNREG: Lipid Hydroxylation

- **Addition of OH to fatty acid → influences cell signalling**
- **Hydroxylation of ARACHIDONIC ACID → HCC CELL PROLIFERATION**
- **LOX/COX → prostaglandins = promotes tumorigenic environment**
- **CYP2E1 → increase of ROS through lipid peroxidation → DNA adduct formation → TP53 mutations and GENOMIC INSTABILITY**

Conclusion

Overall, HDV-HCC pathways favor cell surface pattern recognition pathways which includes PRR activation, causing inflammation driven hepatocarcinogenesis. Meanwhile, BUB1/BUBR1 and AURKA lead to worse prognosis in HDV-HCC. Furthermore, in HBV-HCC, negative regulation of DNA-templated transcription leads to oncogenic activation, and repression of anti-tumor response, while lipid hydroxylation leads to genomic instability.