ARTICLE

Molecular Insights: Gene Expression in HCC and Testicular Cancer among ALD Patients.

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Competing interests
The authors have declared that no competing interests exist.

Abstract

Background: *Alcohol-associated liver disease (ALD) leads to anomalies of hepatocellular carcinoma (HCC), and steatohepatitis. Though tremendous efforts have been made during the past 2 decades, ALD pathogenesis remains obscure. Currently, computational data analysis related to the residues of ALD patients is not widely emphasized, so most attention is needed on differentially expressed genes associated with HCC.*

Methodology*: A comparison between GSM4194985 (Healthy) and GSM4194987 (ALD) was conducted through the GEO database with the accession ID GSE141100 in the form of Raw RNA counts. IDEP analyzed data through bicluster heatmaps for upregulated and downregulated genes for potential effects of ALD on the patients followed by pathway analysis through Reactome.*

Results*: The study revealed the downregulated expression of KCNK15 alongside the upregulation of MLXIPL and ART4 owing to ALD discerning their progression in HCC. As KCNK15 and MLXIPL both are involved in metabolism, their pathway analysis alleged the dysregulation of ion and insulin homeostasis respectively could lead to the progression of HCC. ADH1B downregulation raises the possibility of poor alcohol metabolism, which exacerbates liver damage. Dysregulation of MLXIPL, KCNK15, and ART4 may accelerate the development of HCC. Furthermore, this study suggests that impaired spermatogenesis in ALD patients is associated with overexpression of C5orf58, KCNE1, and AKAP3.*

Conclusion*: This study reveals the inclination of developing HCC in ALD patients based on the differential expression of KCNK15, MLXIPL, and ART4 genes and liver toxicity by ADH1B. The upregulation of C5orf58, KCNE1, and AKAP3 may lead to the defective spermatogenesis that may contribute to the development of testicular cancer owing to the upregulation of C5orf58, KCNE1, and AKAP3 in spermatogenesis.*

Key words: *Alcoholic Liver Disease (ALD), GEO, Hepatocellular Carcinoma (HCC), Testicular Cancer*

Introduction

Alcohol-associated liver disease (ALD) or Alcoholic liver disease is one of the most common chronic liver anomalies in the US, comprising 50% of causes of cirrhosis (Asrani et al., 2021; Devarbhavi et al., 2023; Julien et al., 2020). ALD is not just a single disease, rather it comprises an array of disorders ranging from steatosis to cirrhosis to hepatocellular carcinoma (HCC) and other different alcohol-associated ones like alcohol-associated steatohepatitis (ASH) (Bataller et al., 2022; Crabb et al., 2016). As alcohol is metabolized in the liver and absorbed in the small intestine. The high amount of alcohol could disrupt this metabolization and lead to toxicity, diseases such as fatty liver or alcoholic fatty liver are more concerned in 90% of the individuals who are heavy drinkers, this fatty liver is also named steatosis (Mackowiak et al., 2024). Histological methods diagnose ASH, it is characterized by steatosis, inflammation, and hepatocyte ballooning, in many cases ASH leads to cirrhosis, and individuals having alcohol-associated cirrhosis develop HCC as seen in 3 to 10% of the cases (Mackowiak et al., 2024). In Slovakia, 50% of the people suffer from alcohol-associated hepatocellular carcinoma having cirrhosis in the past, making ALD the most common cause of HCC (Šafčák et al., 2023). According to a study the increasing rate of ALD-associated HCC is common in older males and exclusively in people with cirrhosis in the past, also a decreased rate in the survival of patients is visible in HCC involving ALD v/s in HCC involving non-ALD causes (deLemos et al., 2020).

The high prevalence of HCC in ALD is revealed as the ratio increased from 5.8% to 30.7% in the last 17 years. Furthermore, factors of age, drinking years and sex, preferably male sex were associated with this increased risk of HCC (Chang et al., 2024).

Interindividual as well as progression of the disease differences shows that there is an involvement of genetic factors contributing to the development of HCC by ALD. Genome-Wide Association Studies (GWAS) have shown variants like *I148M PNPLA3, TM6SF2*….. etc lined with the severity of the disease and toxicity. Moreover, several variants are also present related to genes associated with insulin resistance, glucose metabolism, oxidative stress, and more (Choudhary & Duseja, 2021). The metabolites and other by-products formed during the alcohol mechanism contribute majorly to liver toxicity. The first step in alcohol oxidation is Alcohol dehydrogenase (ADH), converting alcohol to acetaldehyde. It has different forms, the major one of ADH involved in alcoholic metabolism is *ADH1B*. Though its role is involved in the elimination of ethanol, an excessive amount of alcohol could disprove its function as the formation of toxic metabolites starts (Di et al., 2021) and this further leads to various liver anomalies involving HCC. K+ serves as a biomarker for the detection of HCC, and they have also been studied to be involved in the proliferation, migration, and further invasion of these tumorous cells during aberrant expression (Chen et al., 2023). Most of the genes involved in metabolism contribute to HCC, such as glucolipid metabolism proteins, specifically from the *MXL* family are taken into consideration in this regard (Chang et al., 2023). As HCC is most common in older males, this could mean that ALD might contribute to the disrupted reproductive capabilities of the individual, such as in spermatogenesis or sperm motility, nevertheless, increased research is needed to shed the light on this scenario.

Methodology

For the analysis of dysregulated genes, credentials were made that sample should be taken as of GSE ID in the form of Raw RNA Counts having *Homo sapiens* as a model organism and expression profiling by high throughput sequencing to generate close to accurate results.

The accession ID number GSE141100 involving 39377 genes ID was taken from the National Centre for Biotechnology Information OR NCBI (https://www.ncbi.nlm.nih.gov) and analyzed on Gene Expression Omnibus OR GEO (https://www.ncbi.nlm.nih.gov/geo/) to reconfirm the credentials. The sample was from Liver, specifically from hepatic stellate cells downloaded in the CSV format that was further converted to editable form in the excel. The sample involved healthy and ALD individuals in the form of various sets. Every set was searched one by one on the GEO database to affirm the status of healthy and ALD individuals and a total of 8 healthy sets and 6 ALD sets were recognized. From those sets, GSM4194985 was taken for Healthy individuals, and GSM4194987 was for ALD-positive individuals on a separate Excel sheet in the form of raw RNA counts having 3 columns for gene IDs, interaction and target. The total number of individuals involved in the Control and Experimental respectively were of the same number i.e. 39376. The data was then uploaded and analyzed for upregulated and downregulated genes through the bicluster correlation feature of iDEP 2.0 (http://bioinformatics.sdstate.edu/idep/) software available online. Healthy individuals were taken as the interaction and ALD individuals as the Target ones, with the same Gene IDs .From the heatmaps generated by the bicluster feature, by analyzing cluster 2, only important genes of metabolism, alcohol oxidation, and spermatogenesis were targeted. For pathway analysis of oxidative genes, the Reactome Pathway Database (https://reactome.org) was used and results were further analyzed through available literature.

Results and Discussion

Cluster 2 of GSM4194985 and GSM4194987 revealed 94 genes correlated across interaction and target specifically. From those, genes dysregulated in the target for metabolism and spermatogenesis were selected. The heatmaps chosen were based on the BCCC method, which is successful as genes were expressed according to a condition that was in that target i.e. ALD.

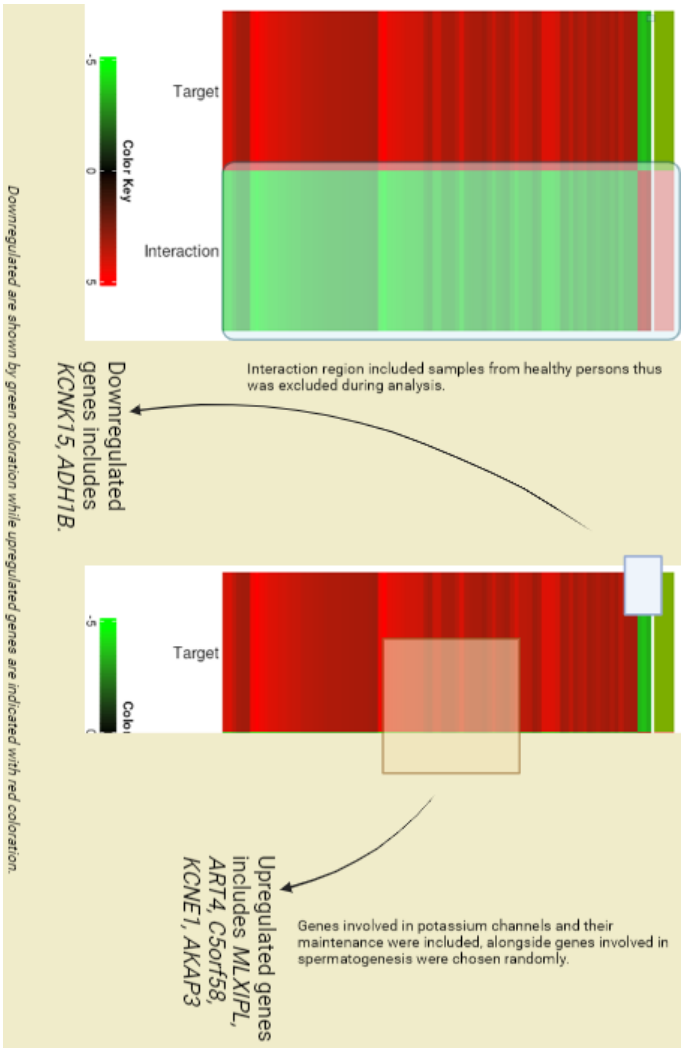

Figure 1: The Schematic diagram shows the choice and selection of upregulated and downregulated genes from bicluster features of iDEP. The red colored indicates the upregulated genes, and green color indicates the downregulated genes. The interaction was studies between healthy and ALD samples labeled by interaction and target respectively.

Identified Genes and their function

As the study focused on the potassium channels involved in HCC, only a few genes were chosen to establish a connection. *KCNK15* and *MLXIPL*, along with *ART4* and ALD, were revealed to be involved in the progression of HCC.

Upregulated genes:

MLXIPL from the *MXL* family of lipid oxidizers was upregulated, and *ART4* which serves in ADP ribosylation having roles in stress responses was also upregulated, all these genes are involved oxidation and metabolism in general. *C5orf58*, *KCNE1*, and *AKAP3* were also overexpressed in the target, *C5orf58* works as a prognostic marker in HCC and has a strong expression in male testis. *KCNE1* works as a potassium gates ion channel, developing its role in HCC. Furthermore, *AKAP3* is one of the major genes involved in normal sperm function, specifically regulation of motility and capacitation was overexpressed.

Downregulated genes:

KCNK15 which serves as a potassium channel was downregulated, *ADH1B* is useful when it comes to the oxidation of alcoholic products and healthy liver metabolism, its downregulation could lead to a serious situation when much alcohol consumption raises the possibility of poor alcohol metabolism, which exacerbates liver damage. This was one of the major genes emphasized to have a strong role in HCC, as described in the pathway below.

The pathway is taken from the KEGG database showcasing the downregulated effects of *alcohol dehydrogenase (ADH)* leading to its inability to oxidize acetaldehyde that may lead to high buildup of acetaldehyde which contributes to the disruption of *AMPK* pathway leading to decreased fatty acid oxidation thus involving in the high risk of HCC through the development of hepatic steatosis

Conclusion

Taken together these findings It can be concluded that differential expression of *KCNK15, MLXIPL, ART4* and *ADH1B* can contribute to development of ALD that could further lead to development of HCC along with development of the testicular cancer owing to the upregulation of *C5orf58*, *KCNE1*, and *AKAP3* in spermatogenesis.

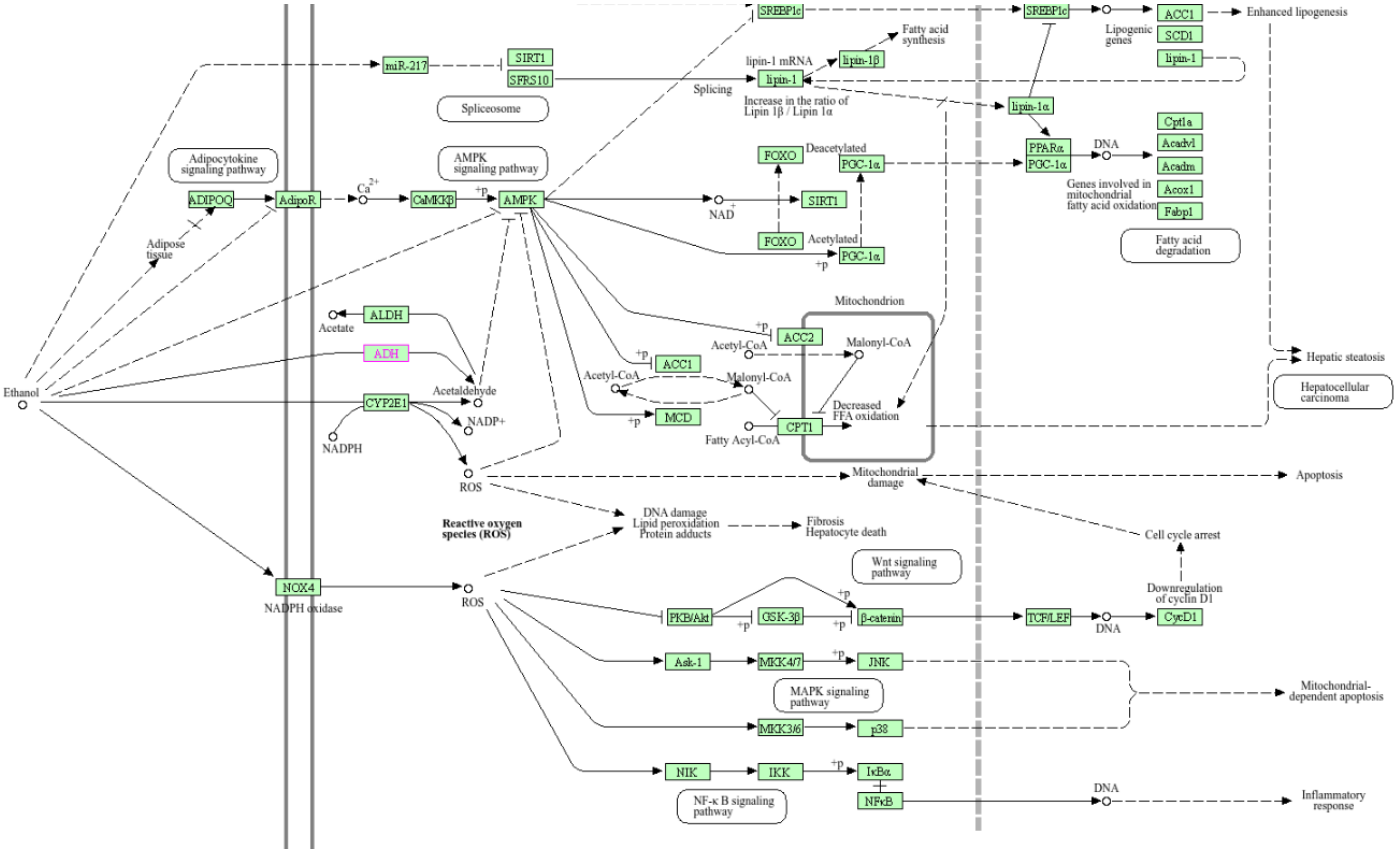

Figure 2: Disrupted ethanol oxidation pathway demonstration by KEGG Database

Author contributions

HF and NS led the study conception, design, including the identification of key molecular pathways. HF, HHM and HA contributed to data collection and performed analysis. HF, HHM and HA contributed to manuscript writing and revising under the supervision of NS. All authors reviewed and approved the final draft for publication.

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