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Short Communication

Unveiling the impacts of metformin on hepatocellular carcinoma: A bioinformatic exploration in cell lines

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Abstract

The most common type of liver cancer is hepatocellular carcinoma (HCC), accounting for 75–85% of cases. Despite its associated side effects, sorafenib remains the standard treatment for HCC. Given the critical need to improve therapeutic efficacy while minimizing adverse effects, alternative drugs must be thoroughly investigated. Numerous studies indicate that combining sorafenib with metformin results in a more favorable treatment profile. The aim of this study was to employ bioinformatics methodologies to elucidate the molecular pathways and genetic underpinnings of metformin's efficacy in HCC treatment. Genes associated with metformin and its action against HCC (Huh-7 and HepG2 cells) were acquired from the NCBI-GEO data collection by utilizing pre-determined keywords. Subsequently, pathways implicated in metformin-mediated HCC treatment were analyzed through the Kyoto Encyclopedia of Genes and Genomes (KEGG). Our analysis revealed the involvement of multiple pathways, with metabolic pathways implicated in 80% of the total cases. Neurodegenerative pathways were involved in only around 60% of the total cases. These findings align with the multifaceted mechanisms of metformin's action, encompassing adenosine monophosphate-activated protein kinase activation, apoptosis induction, insulin regulation, anti-inflammatory responses, and modulation of cell proliferation. This comprehensive investigation sheds light on the intricate molecular landscape underpinning metformin's therapeutic efficacy in HCC, thereby informing potential avenues for optimizing treatment strategies.

Keywords: Bioinformatics, drug repurposing, genetic pathway, HCC, metformin

Introduction

Liver cancer ranks as the second leading cause of global cancer-related deaths [1]. Asia and Africa reportedly have the highest incidence rate of liver cancer [2]. Hepatocellular carcinoma (HCC), the most common type of liver cancer, represented 75–85% of all cases and gradually rose in terms of incidence and mortality [3]. Globally, HCC was estimated to have over 830,200 deaths in 2020, with less than 50% mortality rate within two years of diagnosis and less than 10% of the 5-year survival rate [4,5]. The most common etiologies of HCC include chronic liver damage

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caused by hepatitis B or hepatitis C virus infections, alcoholic liver cirrhosis, and non-alcoholic fatty liver disease (NAFLD) [6]. Individuals with active hepatitis C virus infection have been reported to be 15–20 times more likely to acquire HCC [7]. HCC is a type of liver cancer originating from hepatocytes, the primary cells in the liver. The pathogenesis of HCC involves a sequence of cellular alterations and damage to hepatocytes, culminating in the formation of a malignant tumor [8].

The therapeutic approaches for HCC vary depending on the stage of the disease. Hepatic resection and radiofrequency ablation (RFA) are two key treatment options for early-stage HCC, while sorafenib is the standard treatment for advanced HCC [9]. Sorafenib and lenvatinib are two primary conventional medications used to treat HCC. Compared to lenvatinib, sorafenib has a more established role in clinical practice and is, therefore, more commonly utilized [10]. Treatment outcomes, on the other hand, are often disappointing. The median overall survival with sorafenib therapy is around 8 to 11 months in patients with advanced HCC [11]. Sorafenib is a tyrosine kinase inhibitor that can target various pathways by interfering with numerous kinases, including the mitogen-activated protein kinase system, the vascular endothelial growth factor signaling pathway, and the platelet-derived growth factor signaling pathway [12]. However, compared to the placebo group, sorafenib only provides a few months of increased median survival in individuals with advanced HCC. Sorafenib can cause acneiform rash, diarrhea, hypertension, and fatigue [13].

In addition, to sorafenib administration, metformin has been used as an adjuvant option for HCC therapy. Metformin has been studied for its anti-cancer properties; some studies have found that metformin is related to a lower risk of HCC in people with diabetes [9]. Metformin may inhibit the activation of extracellular signal-regulated kinases 1 and 2 (ERK1/2) and c-Jun n-terminal kinases 1 and 2 (JNK1/2), two key actors in carcinogenesis and proliferation in HCC. Metformin is able to inhibit the expression of urokinase-type plasminogen activator (uPA) and matrix metallopeptidase 9 (MMP-9), whose expression in HCC is linked with a poor prognosis [14]. Furthermore, metformin's ability to decrease glucose and sensitize insulin may inhibit the growth of premalignant hepatic lesions that thrive in high-glucose or insulin environments. Importantly, several studies have shown that metformin can suppress tumor development by activating the adenosine monophosphate-activated protein kinase (AMPK) pathway, which helps regulate cellular energy balance and inhibits the mechanistic target of rapamycin (mTOR) signaling pathway, leading to reduced cell proliferation [15]. Combining modest doses of metformin with sorafenib successfully blocked critical pathways associated with HCC development and treatment resistance and decreased tumor growth in mice [9]. A meta-analysis further supports this combination therapy, demonstrating that metformin significantly prolongs the survival of HCC patients with type 2 diabetes (T2D) after curative treatment [16,17].

The efficacy of metformin monotherapy, particularly in the context of HCC, is still being studied [16]. Given that most cancer treatments rely on combination therapies to achieve optimal therapeutic effects, our study explored the potential of metformin as an adjuvant therapy. We employed bioinformatics techniques to investigate the pathways and genes associated with metformin treatment in HCC. While earlier studies have demonstrated the anti-tumor effects of metformin, the aim of this study was to provide a deeper understanding of the molecular mechanisms and potential gene targets influenced by metformin in the context of HCC. This approach could reveal new insights and therapeutic strategies that have not been extensively explored in earlier studies.

Methods

Data collection and dataset selection

The NCBI-GEO website (https://www.ncbi.mlm.nih.gov/geo) provided the data used in this investigation. The initial search was conducted using the keywords "metformin" and "cancer" to identify relevant datasets. The aim of this study was to gather information about whether metformin has a role in the treatment of HCC and investigate important therapeutic pathways of metformin in HCC cells. After beginning the search with metformin, we used the “human organism” dataset. Following that, RNA sequencing studies were conducted, followed by gene

expression. Seven datasets containing the phrases "metformin" and "cancer" were identified during data analysis.

The seven datasets we acquired illustrate how metformin affects gene expression in various cell types (**Figure 1**). Consequently, several datasets that had no relevance with metformin and liver cancer or were only related to other types of cancers (breast cancer, colorectal cancer, pancreatic cancer, prostate cancer, lung cancer, ovarian cancer, and leukemia) were eliminated, leaving the remaining two datasets. GSE190076 and GSE208245 were the two datasets selected for the study. The terms "gene expression" and "human" were used to extract these two datasets from filters. GEO explains that GSE190076 data refers to the data derived from earlier research, namely the mRNA profiles of Huh-7 cells treated with metformin and untreated for 48 hours [18]. Meanwhile, the GSE2028245 dataset contains a comparative gene expression profiling study of RNA-seq data for HepG2 cells stimulated with metformin, imeglimin, 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR) [19]. The p-adj value was computed, and a value of less than 0.05 was considered statistically significant.

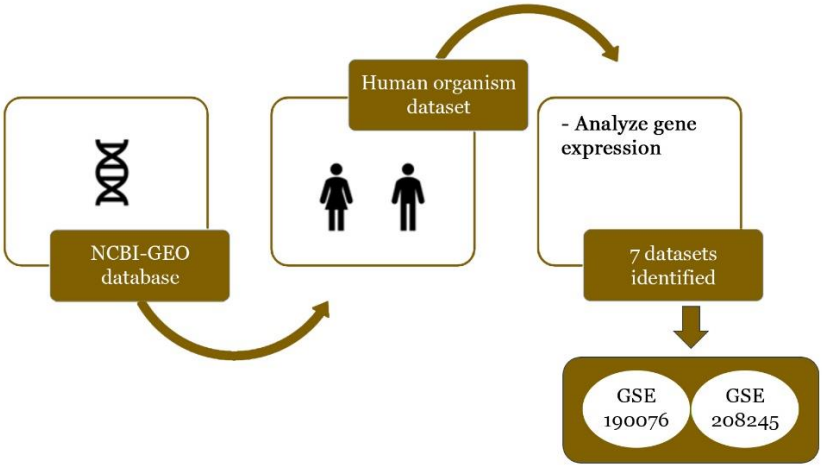

Figure 1. A schematic diagram illustrating the methodology. Seven datasets related to "metformin" and "cancer" were identified and analyzed, focusing on RNA sequencing and gene expression in human cells. Out of the seven datasets, only two datasets remain: GSE190076 and GSE20845

Data and pathway analysis

A Venn diagram was generated to see whether the two data points intersect. A total of 7,464 genes were obtained from the Venn diagram (https://bioinformatics.psb.ugent.be), which were then subjected to pathway analysis using the Kyoto Encyclopedia of Genes and Genomes (KEGG) on ShinyGO 0.77 (http://bioinformatics.sdstate.edu/go/). KEGG was used to analyze and interpret the biological pathways associated with the identified genes, mapping their expression data to understand their roles in liver cancer and the effects of metformin. The KEGG involves several pathways, including the metabolic, endocytosis, and cancer-related pathways.

Gene ontology analysis

The shared genes were further analyzed using Gene Ontology (GO) analysis on ShinyGO 0.77 with “human” as the selected species. This analysis provided a visual representation of the molecular pathways through which metformin impacts HCC. We performed statistical tests, including the false discovery rate (FDR), to adjust *p*-values and mitigate false positives. A lower FDR value indicates more reliable findings.

Results

Differential gene expression analysis of hepatocellular carcinoma (HCC) using the gene expression omnibus (GEO) database

The Gene Expression Omnibus (GEO) database was used to collect transcriptomic data on metformin for HCC. Data was extracted from GEO databases (GSE190076 and GSE208245). The

GSE190076 dataset shows the effect of metformin on HCC Huh-7 cells after 48 hours post-treatment. The GSE208245 dataset examines metformin's, imeglimin's, and AICAR's effects on hepatocytes and the gene expression in cultured human hepatoma HepG2 cells.

Volcano plots visualizing significant differences in gene expression between GSE190076 and GSE208245 datasets are presented in **Figure 2.** The volcano plot provides an overview of gene expression in the genes common to GSE 190076 (shown by blue) and GSE 208245 (represented by red). The gene expression in GSE190076 is more abundant compared to GSE208245. In **Figure 2B**, the gray color represents the genes with no significant change of expression. There were 11,432 and 11,316 genes in the GSE190076 and GSE208245 datasets, respectively.

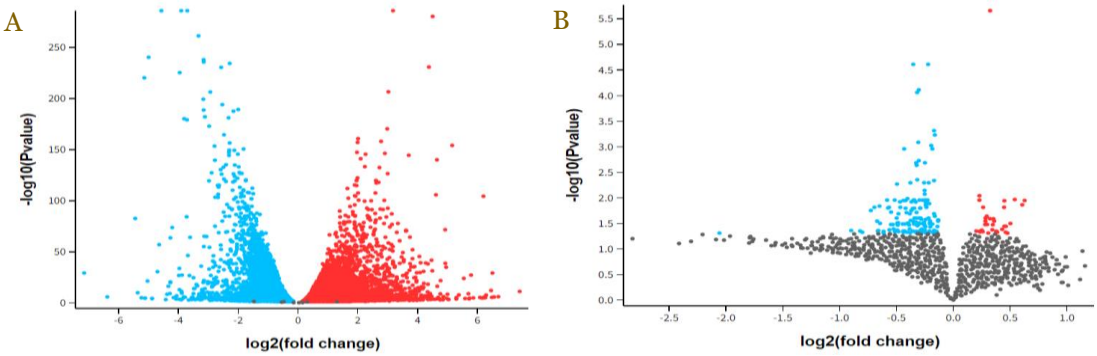

Figure 2. Differential gene expression in hepatocellular carcinoma (HCC) cells affected by metformin in different cancer cell lines: Huh-7 cells from the GSE190076 dataset (A) and HepG2 cells from the GSE208245 dataset (B). The blue dots represent genes significantly affected by metformin treatment compared to the control treatment (sorafenib) in Huh-7 cells, while the red dots represent genes significantly affected by metformin, imeglimin, and AICAR treatments against the HepG2 cells. Genes upregulated in GSE190076 are shown in blue, genes upregulated in GSE208245 are shown in red, and genes with no significant change in expression are shown in gray. Positive log2(fold change) values indicate upregulation in gene expression, while negative values indicate downregulation. The y-axis represents the -log10(p-adj) value, which shows the statistical significance of the observed changes in gene expression.

A total of 7,464 cross-over genes are presented in **Figure 3**. Analysis using ShinyGO revealed the functions of the shared genes. These 7,464 genes were involved in how metformin affects HCC cancer cell lines. Up to 3,968 genes from the GSE190076 dataset and 3,852 genes from the GSE208245 dataset were unique and did not intersect.

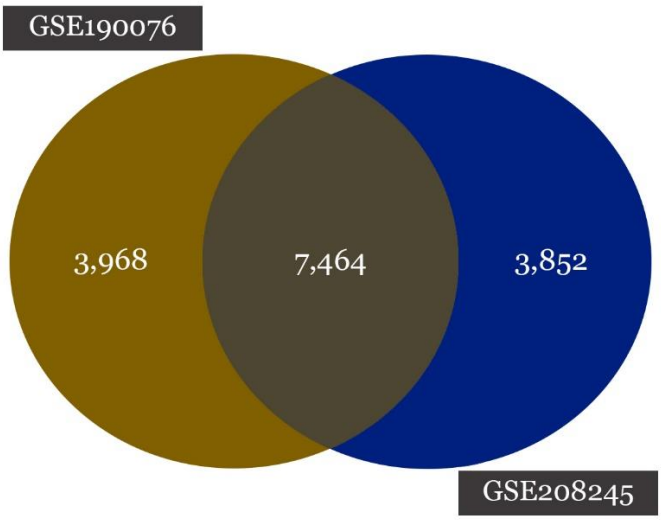

Figure 3. Venn diagram illustrating the overlap between genes in the GSE190076 and GSE208245 datasets, revealing 7,464 intersecting genes. These shared genes were further analyzed using ShinyGO to investigate their roles in the effects of metformin on HCC. Data were obtained from the Gene Expression Omnibus (GEO) database.

Molecular pathways of metformin therapy in HCC

The pathways involved in the treatment of HCC by metformin are presented in **Figure 4**. Our findings demonstrated that the metabolic pathway is the one that most affects metformin treatment when used as an HCC therapy. Based on the p-adj (FDR) value, the metformin pathway image -log10 (FDR) scale indicates the degree of statistical significance of the pathway analysis. The metabolic pathway had the highest -log10(FDR) values: 20 points.

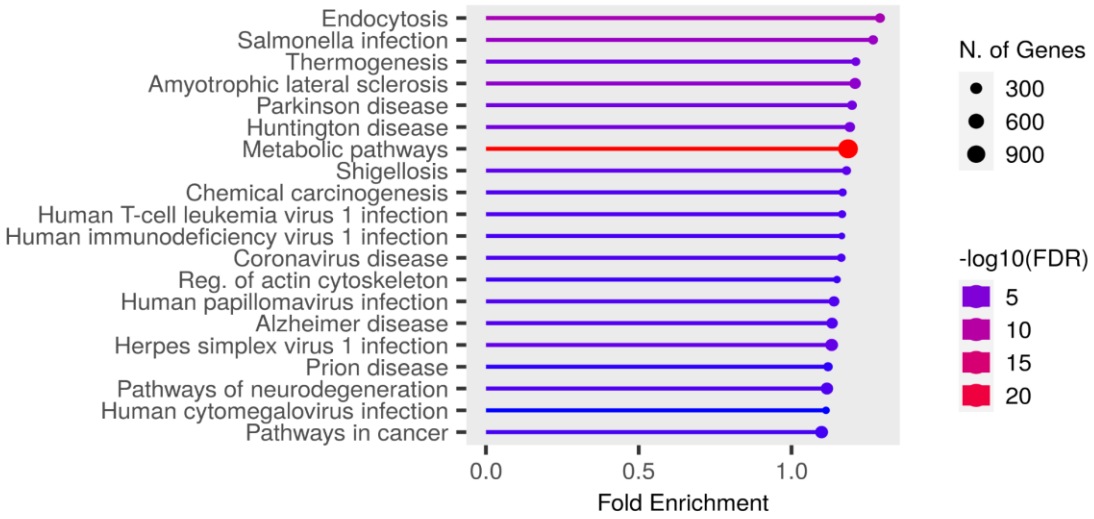

Figure 4. Pathways of metformin therapy in targeting hepatocellular carcinoma (HCC). The metabolic pathways are indicated in red, signifying strong enrichment with a high number of implicated genes. The pathways are arranged according to their fold enrichment. The color gradient indicates the significance level, expressed as -log10(FDR), and the size of the dots indicates the number of genes linked to each pathway.

We analyzed the role of the role of genes identified through KEGG pathway analysis and found that many are involved in the development of cancer (**Figure 5**). Metformin influences multiple complex pathways associated with cancer. Key genes involved in both the metabolic and cancer-related pathways include *PRKAA1/2* (AMPK), *MTOR* (mTOR), *PIK3CA/B/D* (PI3K), and *AKT1/2/3* (AKT). These genes, highlighted in red, are crucial in regulating metabolic processes and are significantly influenced by metformin. Specifically, metformin inhibits mTOR signaling and activates AMPK, both of which are essential for controlling cell division and metabolism. This interaction explains why metformin is an effective treatment for HCC: it disrupts cancer cell growth and survival by targeting these critical metabolic pathways.

Discussion

Our present study reveals that metabolic pathways play a crucial role in the efficacy of metformin therapy for HCC. The data analyzed were derived from Huh-7 and HepG2 cells. Huh-7 cells were chosen for their resemblance to liver cells in terms of morphology, often exhibiting a polygonal or spindle-shaped appearance. These cells are known for their higher proliferation rates and variable sensitivity to certain drugs. The use of Huh-7 cells is thus justified by their relevance to liver cancer and their ability to reflect some aspects of drug response observed in more complex systems [16]. In the second volcano plot, variations in gene expression are observed due to the impact of imeglimin on mitochondrial activity compared to metformin. Metformin and imeglimin are distinct antidiabetic drugs with different mechanisms: metformin primarily activates AMPK, while imeglimin enhances mitochondrial function by activating complex I of the electron transport chain (ETC) [20]. This difference is why imeglimin is used as a comparator to understand metformin's pathway in HCC.

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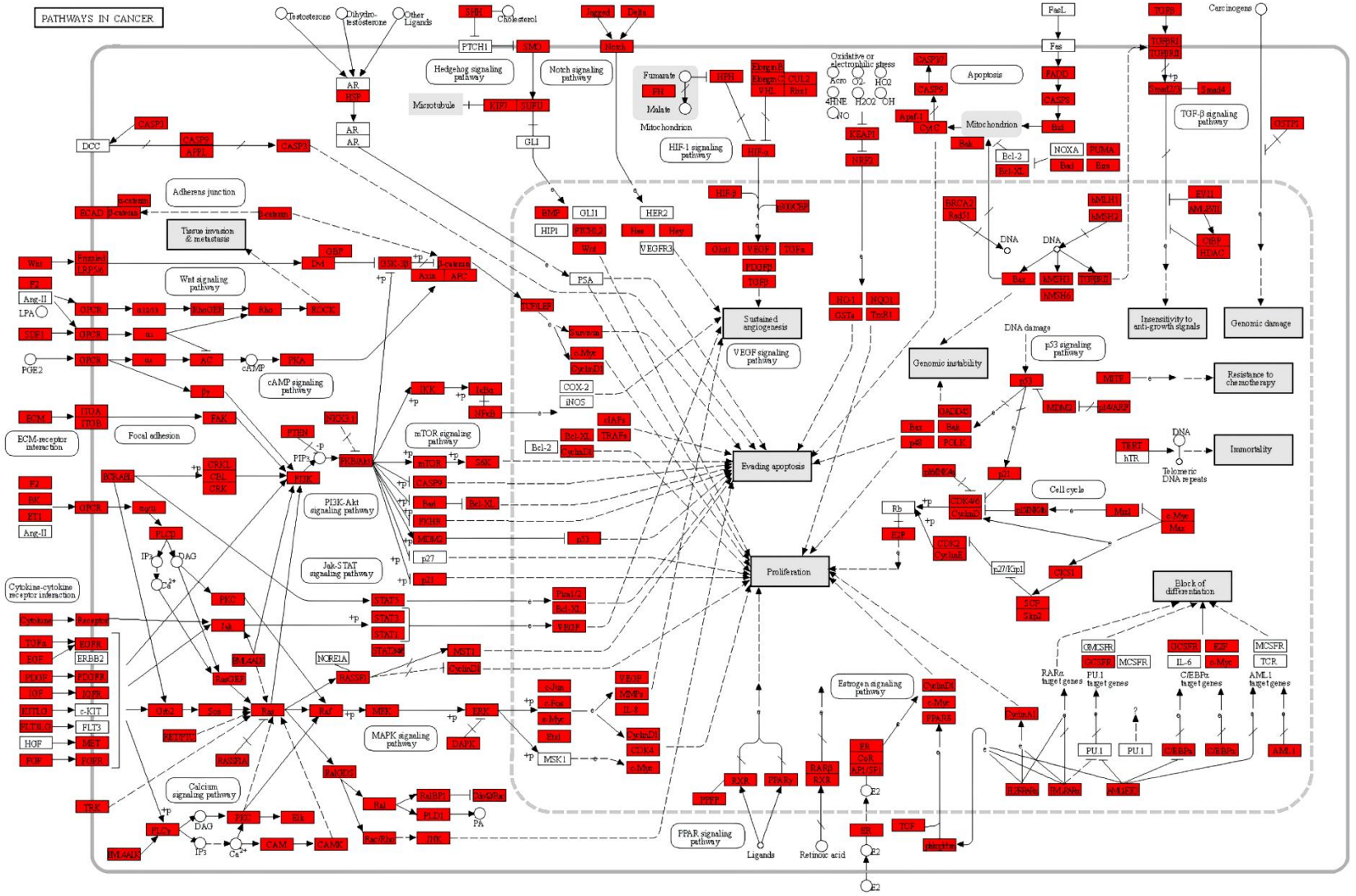

Figure 5. Pathways in cancer. Figure license granted by KEGG [30]. The flow diagram describes the relationship between several genes involved in cancer and the activity of metformin in several cancer-related pathways. The red box in the picture emphasizes the p53 gene, which is implicated in the mTOR signaling pathway (white box). The grey box labeled "Evading Apoptosis" depicts the consequences associated with each signaling route. The arrows depict the directional flow of various pathways, demonstrating how activating or suppressing certain genes such as p53. The importance of these pathways in cancer progression and the potential influence of metformin.

The pathway analysis in the present study suggests that metformin's effect on metabolic pathways, particularly through AMPK activation, plays a key role in HCC treatment. AMPK activation inhibits ATP-consuming pathways like fatty acid synthesis, reducing energy availability for cancer cell proliferation. This also affects lipid metabolism, limiting resources for cancer progression. Metformin-induced AMPK activation can also promote autophagy and cancer cell death [21]. Key genes involved in these processes include *PRKAA1/2* (AMPK), *MTOR* (mTOR), *PIK3CA/B/D* (PI3K), and *AKT1/2/3* (AKT), which are crucial for regulating metabolic processes and are impacted by metformin [22]. In the present study, we found that the mTOR (*MTOR*) gene was impacted by metformin. Subsequently, the *TSC1/2*-inhibited Ras homolog gene enriched in brain (*RHEB*) prevents mTORC1 activation. The suppression of mTOR action could occur concomitantly with the downregulation of *PRKAA1/2* expression by metformin [23]. As suggested by a previous study, metformin could suppress mTOR signaling, decreasing cancer cell growth [24].

Our present study also revealed the mechanism of metformin's effect on the insulin-like proliferation factor 1 (IGF-1) pathway. This pathway regulates the survival and proliferation of cancer cells [25]. The Forkhead Box O (FOXO) is a vital transcription factor that regulates cell survival and proliferation [26]. Previous studies have shown that metformin can limit the growth of different types of cell cancers, including prostate and colorectal cancer, by affecting AMPK and insulin response, survival, and proliferation [27,28].

Another implication of metformin, based on the present study, is its regulatory activity on cell cycle and apoptosis. Metformin administration may cause cancer cells to enter a cell cycle arrest, stopping unchecked growth and division. According to a previous study, metformin has been shown to induce apoptosis, thereby reducing the survival of HCC [24]. In our study, metformin was found to modulate the expression of several genes involved in apoptotic pathways, including *TP53*, *BCL2*, *BAX*, *BAD*, *FAS*, and *TNF*. B-cell CLL/lymphoma 2 (*BCL2*) is an anti-apoptotic gene that inhibits the apoptosis pathway. Metformin was suggested to downregulate *BCL2* expression in HCC. Meanwhile, the *FAS* gene encodes the Fas cell surface death receptor, which mediates the extrinsic apoptotic pathway [29]. Tumor necrosis factor (*TNF*) is a cytokine that regulates the apoptosis process [29].

The findings herein present valuable insights into the molecular mechanisms underlying metformin's therapeutic effect against HCC. In line with a previous study, metformin was shown to reduce chronic inflammation associated with HCC development and enhance anti-tumor immune responses by modulating the tumor microenvironment [31]. Given that HCC is the leading cause of death among liver cancers and is associated with a poor prognosis in nearly all cases, these findings are particularly significant [32]. However, it is crucial to acknowledge the limitations of our in-silico analyses, emphasizing that comprehensive experimental validation is essential to translate these findings into clinically relevant strategies.

Limitations of this study include the absence of clinical data on metformin use for HCC therapy, as the research focused exclusively on Huh-7 and HepG2 cells. This is particularly significant because HCC is a heterogeneous disease with diverse genetic and molecular subtypes, meaning the findings may not represent the full spectrum of HCC. Additionally, the analysis relied solely on predefined pathways within the KEGG database, which may not fully capture metformin's potential efficacy in treating HCC in humans. Further clinical investigations are required to validate these findings in a broader human context.

Conclusion

Findings from this study suggest that metformin can regulate multiple pathways involved in early cancer development, underscoring its potential efficacy in HCC management. Though our data are promising, translating these bioinformatic insights into clinical practice demands thorough validation of designed clinical trials. Comprehensive clinical trials are imperative to elucidate the full therapeutic potential of metformin in HCC therapy.

Ethics approval

Not required.

Acknowledgments

Nothing to declare.

Competing interests

The authors declare no conflicts of interest regarding the publication of this article.

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Underlying data

Derived data supporting the findings of this study are available from the corresponding author on request.

How to cite

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