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Original paper

A comprehensive investigation on liver regeneration: a meta-analysis and systems biology approach

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Abstract

Aim of the study: Liver regeneration is one of the essential fields of regenerative medicine as a branch of tissue engineering and molecular biology that draws global researchers' attention. This study aims to conduct a systematic review and meta-analysis on the high-throughput gene expression microarray dataset of liver regeneration on the NCBI-GEO database to identify the significant genes and signaling pathways and confirm the genes from literature studies on associated diseases.

Material and methods: We thoroughly searched the NCBI-GEO database to retrieve and screen the GEO microarray datasets' contents. Due to the inclusion of different species in eligible GEO datasets in the meta-analysis, the list of significant genes for the random-effects model were identified. Moreover, we carried out detailed gene analyses for three main gene ontology components and the KEGG signaling pathway. Furthermore, we investigated the possibility of genes' association with liver cancer through the Kaplan-Meier plot.

Results: The random-effects model from six eligible GEO datasets identified 71 genes with eight down-regulated and 63 up-regulated genes. The target genes are involved in various cellular functions such as cell proliferation, cell death, and cell cycle control. Finally, we noted that 58 out of 71 genes are associated with different types of diseases related explicitly to other liver and inflammation diseases.

Conclusions: The current study assessed various GEO datasets at the early stages of liver regeneration with promising results. The present systematic review and meta-analysis results are beneficial for future novel drug design and discovery specifically for patients in the liver transplantation process.

Key words: liver regeneration, meta-analysis, systematic review, systems biology, gene.

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Introduction

The liver is one of the magic metabolic organs in the live body. It is mainly composed of hepatocytes in hemostasis and normalizes different metabolisms ranging from carbohydrates to lipids [1-3]. Several factors can cause liver injury, such as accidental or intentional ingestion of toxins, surgery, or transplantation processes [4, 5]. The liver is known to have a high capacity in the regeneration of its damaged tissues compared to other body organs [6]. Additionally, regenerative medicine, as one of the interdisciplinary translational science fields attracting the attention of the world's research community, uses tissue engineering and stem cell therapy techniques in replacing the injured organ with an artificial organ, which is in a direct relationship with the immune system [3, 7]. Through different original or review literature articles, the molecular and functional mechanisms have been explicitly investigated on animal models (e.g., rats and mice) and *Homo sapiens* to identify the significant genes and signaling pathways involved in liver regeneration [6, 8].

The processes of development, repair, and regeneration of the liver are vital and complex in several aspects

deserving to be studied in molecular mechanisms and epigenetics [9, 10]. Moreover, various therapeutics for liver regeneration have been proposed, including cyclosporine A, tri-iodothyronine, mesenchymal stem cell infusion, transforming growth factor β (TGF-β), interleukin (IL)-1, nuclear factor κB (NF-κB), tumor necrosis factor α (TNF-α), IL-6, glutamine, and amino acids, with simulating and inhibiting effects [10]. Despite its high regeneration capacity, it may be affected by severe and advanced liver diseases. That sometimes can be repaired using liver progenitor cell-driven liver regeneration; however, this approach may not be useful in progressive conditions and needs careful and promotional considerations for future designing and developing tactics [11].

The reports showed that the pattern of gene expression levels in liver regeneration after an injury is unique. Different samples are affected by a specific gene with its interconnection with other genes. The other gene expression pattern types can also affect one molecule on other regenerated liver cells, considering the ethanol-treated and parenchyma liver cells. Additionally, the miRNA pattern changes, for example, on male rat miRNA microarray profiles for a 24-hour duration have similar characteristics. Finally, these types of pattern changes can also represent differences in cytokine levels [e.g., interferon γ (IFN-γ) in aged humans].

Hence, we conducted a systematic review and meta-analysis approach to investigate the molecular and functional analyses of liver regeneration of publicly available microarray datasets for humans, mice, and rats. However, some studies in the literature have performed the same analysis on only one type of species. The current research output will present the significant essential genes, gene ontology, and KEGG signaling pathway analyses in liver transplantation. Finally, the identified genes can help develop novel therapeutics and drug design and discovery approaches relating to specifically liver regeneration failures.

Material and methods

Microarray profiling datasets

We searched the National Center for Biotechnology Information for retrieving the target GEO datasets by the Boolean query "hepatectomy OR hepatic systematically" AND "liver regeneration". The included datasets' eligibility was determined based on their titles and contents by having all types of species, liver biopsy tissues, expression types, and platforms with 1-hour duration without age restriction. We omitted those GEO datasets not satisfying the inclusion criterion from further analysis and utilized the included ones for other processes.

Meta-analysis approach

The ExAtlas online web server is a comprehensive and useful tool for performing meta-analysis on GEO datasets on various platforms with a user-friendly interface to import and utilize the preprocessing steps (i.e., normalization and single-factor ANOVA) in the background for comparing the paired samples based on *p*-values and *F*-statistics. Then, the ExAtlas web server processed the imported datasets for the data quality, in which it discarded those samples with a standard deviation (STD) of more than 0.3. Moreover, from conducting the meta-analysis, the final step, the false discovery rate (FDR ≤ 0.05), and fold change ≤ 1.5 were important sets of parameters. The Benjamini-Hochberg procedure was the method used to decrease the significant number of false-positive genes.

Gene ontology and KEGG enrichment analyses of significant genes

DAVID v. 6.8 (Database for Annotation, Visualization, and Integrated Discovery) website was used to analyze three gene ontology (GO) domains (cellular component, biological process, and molecular function) and involved several Kyoto Encyclopedia of Genes and Genomes (KEGG) signaling pathways [12, 13]. Moreover, the *p*-value term for the genes to be considered significant were set to be less than 0.05.

Kaplan-Meier analysis

To further analyze the obtained significant genes from the meta-analysis approach, the Kaplan-Meier methodology was applicable to investigate survival rates of the input genes in liver cancer versus a living period. This process was necessary because the liver regeneration process had an indirect relationship with liver diseases such as liver cancer [14, 15]. To this end, we used the mRNA liver cancer section of kmplot.com to determine the overall survival (OS; $n = 364$), recurrence-free survival (RFS; $n = 316$), progression-free survival (PFS; $n = 370$), and disease-specific survival (DSS; $n = 362$). We used the website's features inducing pathology, patients, and risk factors as default by enabling the plot option to generate high-quality TIFF image output files. Moreover, the significant genes involved in liver cancer in terms of OS, RFS, PFS, and DSS) were indicated by *p*-values less than 0.05.

Results

An overview of the systematic review of the NCBI-GEO database is available in Figure 1. The process is generally useful in applying the inclusion and exclusion criteria for identifying the final eligible GEO datasets. The systematic review procedure revealed 190 datasets based on the Boolean query from which 11 datasets remained after a critical inspection of the titles and contents. On the other hand, we examined the datasets in the ExAtlas online web server for their availability for further analysis. The outcomes showed that only 6 out of 11 GEO datasets had the required information. The remaining five datasets had platforms and experiment types related to being not RNAs. At the end, six GEO datasets were identified including GSE12720 (*n*_control = 21 and *n*_regeneration = 21), GSE15239 (*n*_control = 2 and *n*_regeneration = 2), GSE20427 (*n*_control = 3 and *n*_regeneration = 3), GSE33785 (*n*_control = 6 and *n*_regeneration = 6 with various diets including ethanol, carbohydrates, and high fat), GSE34057 (*n*_control = 2 and *n*_regeneration = 2 with diets including ethanol and carbohydrate), and GSE6998 (*n*_control = 2 and *n*_regeneration = 2) for 1 hour after the liver transplantation period on liver biopsy tissues. The detailed information for the six datasets is available in Table 1. In the next step, after the successful import of six datasets on *Homo sapiens*, rats, and mice as well as data analysis quality analysis with no outliers, the meta-analysis approach resulted in seventy-one genes. The criteria parameters were FC ≤ 1.5 (those genes with FC larger than 1.5) and FDR ≤ 0.05 for an overall number of 72 samples for both control (*n* = 36) and liver transplantation (*n* = 36).

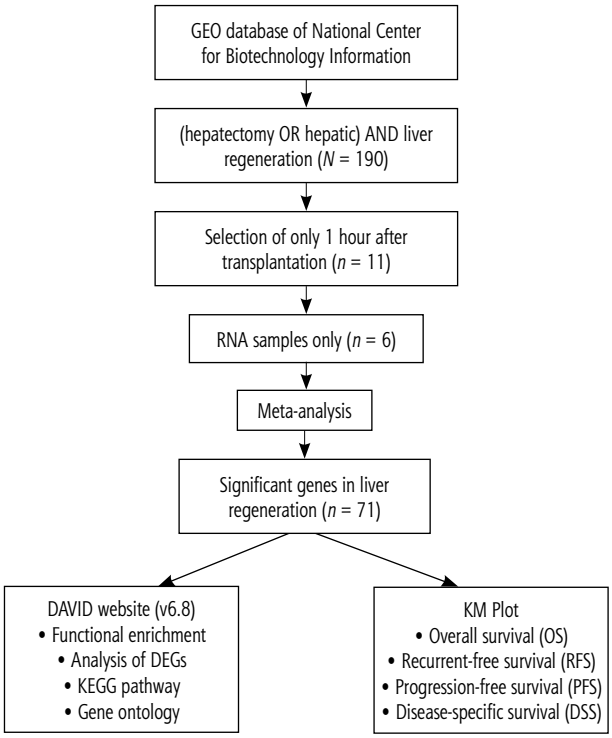

Fig. 1. The systematic review flowchart for applying including and excluding criteria to retrieve the final eligible GEO datasets

Additionally, significant genes among the three species were eligible for analysis in DAVID's bioinformatics web server. A portion of the input genes enriched the BP (e.g., GO:0006366~transcription from RNA polymerase II promoter, GO:0032496~response to lipopolysaccharide), CC (e.g., GO:0005634~nucleus), MF (e.g., GO:0003700~transcription factor activity, sequence-specific DNA binding, GO:0000978~RNA polymerase II core promoter proximal region

Table 1. Detailed information on six GEO datasets eligible for meta-analysis

GEO	Published (PMID)	Species	Platform	Experiment type	Sample type	Source name
GSE12720	19353763	*Homo sapiens*	GPL570	Expression profiling by array	RNA	Liver biopsy
GSE15239	N/A	*Homo sapiens*	GPL570	Expression profiling by array	RNA	Liver
GSE20427	20564353	*Mus musculus*	GPL81 GPL1261	Expression profiling by array	RNA	Liver
GSE33785	22790595 27012785	*Rattus norvegicus*	GPL6247	Expression profiling by array	RNA	Chronic ethanol-fed liver Fed carbohydrate liver Fed high fat liver
GSE34057	22823254	*Rattus norvegicus*	GPL14889	Non-coding RNA profiling by array	RNA	Ethanol-fed liver Ethanol-fed liver
GSE6998	17227769	*Mus musculus*	GPL1261	Expression profiling by array	RNA	Hepatectomy

Table 2. Output of Kaplan-Meier plot for overall survival (OS), *recurrence-free survival* (RFS), progression-free survival (PFS), and disease-specific survival (DSS) rates. The genes with significant *p*-values and no data are highlighted in bold and gray, respectively

Gene ID	OS	RFS	PFS	DSS
ZBED5	**0.003**	0.0691	**0.0045**	**0.0009**
LDLRAD4	No data	No data	No data	No data
LRRC37A2	0.1425	0.0692	**0.0143**	0.1632
ADORA2A-AS1	No data	No data	No data	No data
RTP4	**0.0219**	**0.021**	**0.0063**	**0.037**
DLEU1	0.2554	**0.0108**	**0.0168**	0.0503
FLJ37453	0.2374	0.2816	0.3419	0.2479
SMIM10L2A	No data	No data	No data	No data
BTG2	0.3459	**0.0008**	**0.015**	0.2117
MYC	0.0883	0.1173	0.1623	0.1357
SIK1	**3.7e-5**	0.1546	0.1058	**0.0012**
JUN	0.3599	0.1405	0.2655	0.2081
MIR6732	No data	No data	No data	No data
CXCL8	No data	No data	No data	No data
NOCT	No data	No data	No data	No data
KLF6	0.0782	0.1276	0.1145	0.1881
CCN1	No data	No data	No data	No data
NFIL3	0.1694	0.203	0.2328	0.2092
GDF15	0.0769	0.0534	0.0648	0.2222
SGK1	**0.0116**	0.0731	**0.0343**	**0.0313**
ARL5B	0.0578	0.0694	**0.0102**	0.1265
SLC38A2	0.1796	0.2052	0.1286	0.1061
BIRC3	0.4525	0.2439	0.2981	0.3399
GADD45B	**0.0005**	**0.0231**	**0.0043**	**0.0005**
SDS	0.0169	0.0792	**0.0065**	**0.0427**
TSC22D2	0.128	0.099	0.1211	**0.0324**
ZNF331	**0.0264**	0.2766	0.1639	0.1927
VNN3	0.1836	**0.0104**	**0.0246**	0.0599
MAFF	0.0583	0.4564	0.2274	0.2622
CSRNP1	**0.0071**	**0.005**	**0.0007**	**0.026**
JUNB	0.1361	**0.0103**	**0.004**	**0.0447**
ZFP36	0.0796	0.0571	0.0872	**0.0441**
S100A9	**0.0001**	0.1028	0.057	**0.0005**
THBD	**0.0007**	**0.0035**	**0.0027**	**0.0014**
BHLHE40	0.1656	0.1846	0.2219	0.1462
SDC4	0.0027	0.0177	0.013	0.0358
TCIM	No data	No data	No data	No data
IFRD1	0.0997	0.083	0.1972	0.0727
PTP4A1	0.002	0.2971	0.0712	0.093
CEBPD	0.0022	0.009	0.0066	0.0004
SLC2A14	0.0611	0.2228	0.339	0.1177
WSB1	**0.0209**	**0.017**	**0.0007**	**0.0022**
CCNL1	0.2028	0.2989	0.1965	0.0541
ELL2	**0.0227**	**0.0068**	**0.0223**	0.0849
PIM3	0.2517	0.1098	**0.0192**	0.1525
RETREG1	No data	No data	No data	No data
GPCPD1	**0.0062**	0.1136	0.0876	**0.0062**
ALAS1	**6.8e-5**	0.0555	**0.0112**	**0.0015**
NNMT	0.085	0**.0488**	0.0702	**0.0391**
JUND	**0.0017**	0.2282	0.0537	**0.0007**
NRBF2	0.1633	0.1174	0.0641	0.1889
MAFK	0.3297	0.2452	0.217	0.3273
TIMM23B	No data	No data	No data	No data
LOC100996792	No data	No data	No data	No data
MT1M	0.2659	0.0536	**0.0177**	0.3193
SLC25A33	0.3775	**0.0462**	0.0585	0.284
CEMIP2	No data	No data	No data	No data
USP36	**0.0423**	**0.0165**	**0.0007**	**0.0394**
CEBPB	**0.0087**	0.1607	0.0647	**0.0083**
PNRC1	**0.0273**	0.2189	0.2985	0.0847
MIR636	No data	No data	No data	No data
CXCL3	**0.0013**	0.1966	**0.0297**	**0.0128**
CHAC1	0.0569	0.0704	0.1669	0.1139
NFKBIA	0.0656	**0.0216**	0.081	**0.035**
AKAP12	0.0676	0.2141	0.0991	**0.0259**
DNAJB4	0.0617	0.1582	0.2692	**0.029**
HBG1	0.0004	0.0004	0.0009	**0.0009**
MIR21	No data	No data	No data	No data
BCL2L11	**0.0289**	0.0748	**0.0269**	0.0691
BTG3	0.0967	0.2024	0.1592	0.066
PRR26	No data	No data	No data	No data

sequence-specific DNA binding), and KEGG signaling pathways (e.g., hsa04668: TNF signaling pathway, hsa04380: Osteoclast differentiation).

Finally, the KM plot analysis for the seventy-one genes revealed that only 24 out of 56 genes for OS, 17 out of 56 genes for RFS, 24 out of 56 genes for PFS, and 26 out of 56 genes for DSS are significantly useful for liver cancer (shown in Table 2 and Fig. 2). However, the KM plot website did not analyze fifteen genes as they were not available in its database. That is to say that the

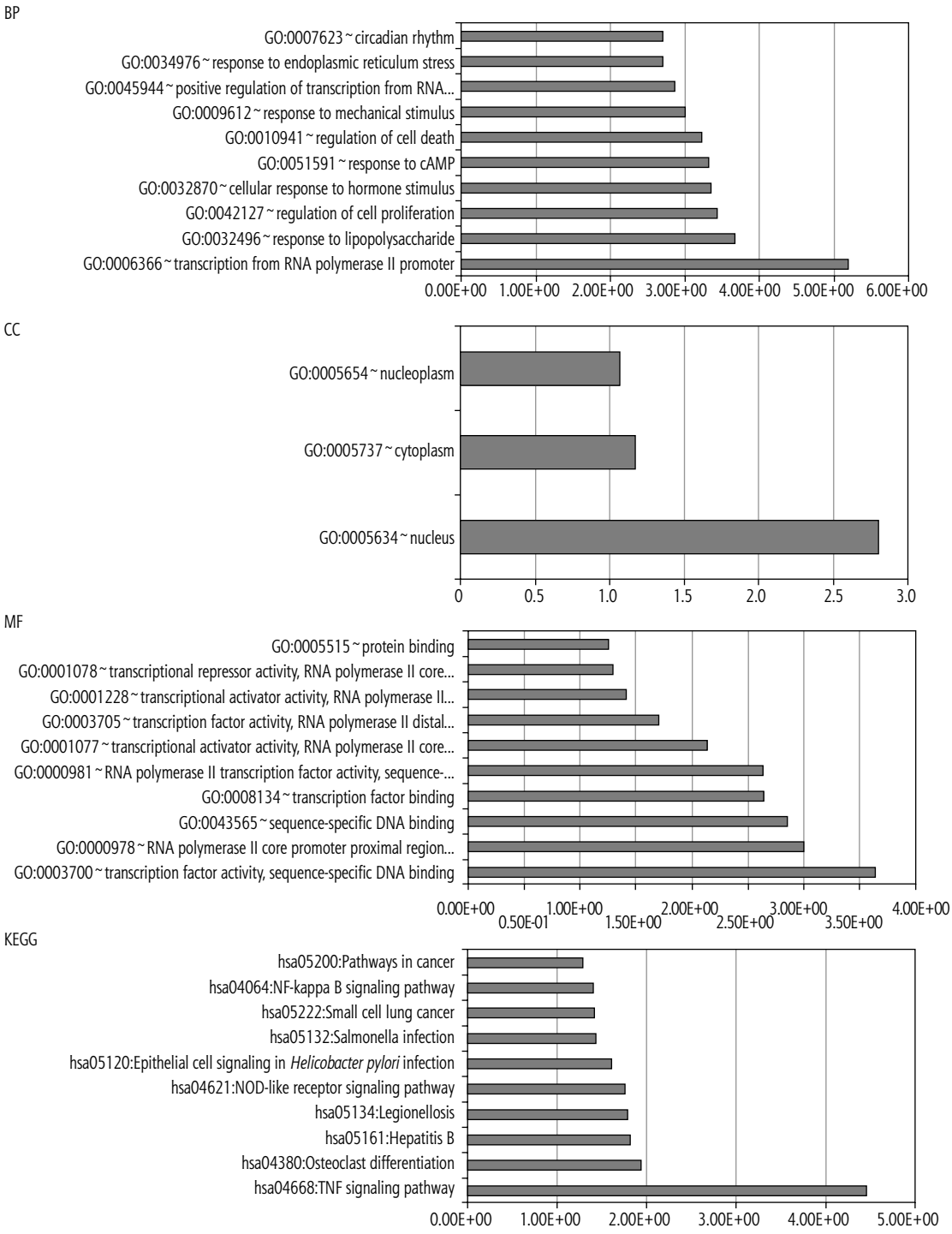

Fig. 2. DAVID's webserver results demonstrated for biological processes (BP), cellular components (CC), molecular functions (MF), and the KEGG signaling pathway enrichment analysis

inhibition of liver cancer may happen after successful liver transplantation.

Discussion

We performed a systematic review and meta-analysis and a systems biology approach on six GEO datasets that remained from screening and analyzing the NCBI-GEO database contents. The outcomes will be beneficial to thoroughly study liver regeneration and the common genes involved in three species (i.e., *Homo sapiens*, mice, and rats). Whether they are present in genetics or signaling pathways involved in liver regeneration, mechanisms are paramount to effec-

Table 3. Identified associated genes with involved signaling pathways of liver regeneration

Pathways	Gene terms
NF-κB	*CXCL8*, *BIRC3*, *GADD45B*, *CXCL3*, *NFKBIA*
MAPK	*GADD45B*, *JUND*
PI3K/AKT	*MYC*, *SGK1*, *BCL2L11*
Wnt	*MYC*, *JUN*
JAK/STAT	MYC
Cellular senescence	*MYC*, *CXCL8*, *GADD45B*, *ZFP36*
TNF signaling pathway	*CEBPB*, *CXCL3*, *NFKBIA*, *BIRC3*, *JUNB*

tively utilize them in clinical experiences. The above issue has also been frequently investigated in both sciences, including biological and clinical fields, considering its complicated structure to find better novel drug design and discovery tactics. The researchers reported that the time required for liver regeneration is twelve weeks. However, in all of the available GEO datasets it was far less than the reported completion of the liver regeneration period (e.g., 0.5, 1, 1.5, 2, 6, 36, and 72 hours) from which for the meta-analysis, the one-hour samples were in common among the GEO datasets [16]. It is worth mentioning that complex process of liver regeneration is composed of three phases: priming, proliferative, and termination phases [17]. In the priming stage, main cytokine-associated pathways such as TNF-α and IL-6 are involved. In the proliferative stage, the complete mitogens epidermal growth factor (EGF), transforming growth factor α (TGF-α), and heparin-binding EGF-like growth factor (HB-EGF) can play significant roles. Meanwhile, the terminative process of liver regeneration has remained unclear. However, some studies have shown that TGF-β family members such as TGF-β1 are enough to end the liver regeneration after 48 h following partial hepatectomy (about two-thirds of the liver) [18]. This period could be enough for obtaining the information to understand the process of liver regeneration in a time-series analysis. Moreover, liver regeneration's early proliferation phase is critical to capturing significant transcriptomic signatures [19].

The meta-analysis results reveal the significant common gene biomarkers among three species playing a vital role in their liver regeneration at the early phase.

We also analyzed the possible direct relationships among genes and their involved diseases using a gene-disease network approach to further discuss the outcomes. DisGeNET (v7.0), a publicly available database web interface, provided a diverse update (June 2020) and curated data bank for retrieving information on genes linked to human diseases using 1,134,942 gene-disease associations [20-22]. We used the "disgenet2r" R package to accurately automate the retrieval procedure of the gene-disease associations (GDAs) using the "gene2disease" command through a working Internet connection [22]. This section provides the required data to discuss the related diseases with the identified significant human genes. Among the reported genes of different illnesses, 58 out of 71 genes were directly consistent with various conditions. These conditions included liver, infection, bile, biliary, blood, measurement, bleed, mental, seizure, cirrhosis, behavior, metabolic, cirrus, cardiologic variants, edema, coronary, vascular, sepsis, and inflammatory. A detailed summary of these GDAs as a collective set of .csv files is available as supplementary material.

As a complex process in *Homo sapiens*, rats, and mice, liver regeneration needs a more clinical and experimental research to understand various biological and molecular aspects of the process for long periods. The liver regeneration core depends explicitly on the cell-cycle proliferation, which activates multiple signaling pathways, including NF-κB, MAPK, PI3K/AKT, Wnt, JAK/STAT, cellular senescence, and TNF signaling pathway [23, 24]. In this regard, the total of fourteen identified associated genes with these signaling pathways, playing an important role in the other liver regeneration phases, are summarized in Table 3. In contrast to the abovementioned accelerating factors, a diverse range of active proliferative-inhibiting liver regeneration pathways are mainly associated with cytokines, oncogenes, gene expression regulation, miRNAs, protein modification, and hormone, metabolism, and pathological factors [25]. The decrease and increase of inhibitor promoters' expression levels may affect different stages of liver regeneration, namely "hepatostat", which requires more studies on inhibitory factors than promoting aspects of liver regeneration [24, 25]. Finally, because cyclin-dependent kinase (Cdks)/cyclin complexes regulate cell-cycle progression, it can be deduced that the main factors, whether they are inhibiting or promoting the process of liver regeneration, occur in the cell cycle [26]. Researchers have reported that the Cdk1 gene contributes to metabolic pathway functions such as cell division and DNA repair [27, 28].

Furthermore, Schmidt and Dalhof reported that α-fetoprotein (AFP) in acute liver failure (ALF) patients could be regarded as a functional marker of hepatocyte proliferation [29]. One of the treatments that can be clinically applied for successful liver surgery is platelet therapy (e.g., thrombopoietin receptor agonists, artificial platelets [30], and freeze-dried platelets [31]). It has a positive protective role for hepatocytes resulting in liv-

er regeneration promotion [23]. Finally, although gene therapy through activating autophagy using mesenchymal stem cells (MSC) transplantation can promote liver regeneration, autophagy suppression may result in functional failure of the liver [32, 33].

However, one of the main limitations of the current systematic review and meta-analysis is the availability of a limited number of GEO datasets for liver regeneration, considering those tracked for extended periods (e.g., six weeks). The next limitation is related to the "experiment type", not supported in ExAtlas due to the microarray platform and non-RNA samples (e.g., genomic in GSE74273). This systematic review and meta-analysis provide in-depth information on liver regeneration's biological and molecular processes common among three species. This study paves the way for future research on liver-related diseases such as acute and chronic liver failure, injury, and transplantation.

Conclusions

Regeneration medicine is a vital field contributing several advantages to the liver regeneration process. We carried out a systematic review and meta-analysis to understand further its biological and functional mechanisms for the process's importance and complexity. Through a systematic review on the NCBI-GEO database, six datasets were eligible for meta-analysis; their organisms include *Mus musculus*, *Rattus norvegicus*, and *Homo sapiens*. The meta-analysis results identified seventy-one significant genes common to three types of organisms. Among them, 27/56, 24/56, 26/56, and 17/56 genes were directly relative to liver cancer for PFS, OS, DSS, and RFS, considering the KM plot (i.e., fifteen genes were not available for KM plot analysis in liver cancer). Moreover, the enrichment analysis of KEGG signaling pathways showed that TNF, NOD-like receptor, and NF-κB signaling pathways were important in liver transplantation. Finally, the involved genes common to three species can predict future drug design and discovery to expedite the liver regeneration process.

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Disclosure

The authors declare no conflict of interest.

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