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Research paper

Inhibition of hepatocellular carcinoma tumorigenesis by curcumin may be associated with *CDKN1A* and *CTGF*

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ABSTRACT

This study aimed to explore crucial genes, transcription factors (TFs), and microRNAs (miRNAs) associated with the effects of curcumin against hepatocellular carcinoma (HCC). We downloaded data (GSE59713) from Gene Expression Omnibus to analyze differentially expressed genes (DEGs) between curcumin-treated and untreated HCC cell lines. Then, we identified the disease ontology (DO) and functional enrichment analysis of these DEGs and analyzed their protein–protein interactions (PPIs). Additionally, we constructed TF–target gene and miRNA–target gene regulatory networks and explored the potential functions of these DEGs. Finally, we detected the expression of *CDKN1A*, *CTGF*, LEF1 TF and MIR-19A regulated by curcumin in PLC/PRF/5 cells using RT-PCR. In total, 345 upregulated and 212 downregulated genes were identified. The main enriched pathway of upregulated genes was the TNF signaling pathway. The downregulated genes were significantly enriched in TGF-beta signaling pathway. In addition, most DEGs were significantly enriched in DO terms such as liver cirrhosis, hepatitis, hepatitis C and cholestasis (eg., CTGF). In the constructed PPI network, CDKN1A and CTGF were the key proteins. Moreover, LEF1, CDKN1A, and miR-19A that regulated CTGF were highlighted in the regulatory networks. Furthermore, the expression of *CDKN1A*, *CTGF*, LEF1 TF and miR-19A regulated by curcumin in PLC/PRF/5 cells was consistent with the aforementioned bioinformatics analysis results. To conclude, curcumin might exert its protective effects against HCC tumorigenesis by downregulating LEF1 and downregulating *CTGF* regulated by MIR-19A and upregulating *CDKN1A* expression.

1. Introduction

Primary hepatic cancer, also known as hepatocellular carcinoma (HCC), is the sixth most common cancer and is considered as the second leading cause of cancer-related deaths worldwide (Berretta et al., 2017). Hepatitis B virus, hepatitis C virus, cirrhosis, hemochromatosis, glycogen storage disease, diabetes, obesity, excessive alcohol consumption, and metabolic diseases are major risk factors for HCC (Benvegnu et al., 1994; Chiaramonte et al., 1999; Berretta et al., 2017). Owing to its late diagnosis, patients with HCC generally have a poor prognosis (Niu et al., 2016). Significant efforts have been taken for treating HCC in recent decades. Nevertheless, the 5-year overall survival rate remains as low as 18% (Kulik and Chokechanachaisakul, 2015). Therefore, a better understanding of the molecular mechanisms underlying the development and progression of HCC is needed to improve its early diagnosis.

Genetic and epigenetic alterations play a crucial role in HCC. In recent years, it has been widely accepted that both genomic instability and somatic mutation play essential roles during the tumorigenesis and development of HCC (Liu et al., 2014; Niu et al., 2016). Due to the

Abbreviations: TFs, transcription factors; miRNAs, microRNAs; HCC, hepatic cancer; DEGs, differentially expressed genes; KEGG, Kyoto Encyclopedia of Genes and Genomes; PPIs, protein-protein interactions; OS, overall survival; NGS, next generation sequencing; MATHT, Multifaceted Analysis Tool for Human Transcriptome; ORA, Overrepresentation Enrichment Analysis; TNF, tumor necrosis factor; ID1, inhibitor of DNA binding 1; LEF1, Lymphoid Enhancer Binding Factor 1; *HABP2*, Hyaluronan binding protein 2; *CDKN1A*, Cyclin Dependent Kinase Inhibitor 1A; *CTGF*, Connective Tissue Growth Factor

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Table 1
The primer sequence for each validated gene.

Primer name	Primer sequence (5′-3′)
GAPDH-rF	TGACAACTTTGGTATCGTGGAAGG
GAPDH-rR	AGGCAGGGATGATGTTCTGGAGAG
U6-hF	CTCGCTTCGGCAGCACA
U6-hR	AACGCTTCACGAATTTGCGT
LEF1-hF	AGAACACCCCGATGACGGA
LEF1-hR	GGCATCATTATGTACCCGGAAT
CDKN1A-hF	TGTCCGTCAGAACCCATGC
CDKN1A-hR	AAAGTCGAAGTTCCATCGCTC
CTGF-hF	AAAAGTGCATCCGTACTCCCA
CTGF-hR	CCGTCGGTACATACTCCACAG
hsa-miR-19a-RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACTCAGTT
JH-hsa-miR-19a	GCGCTGTGCAAATCTATGCAA

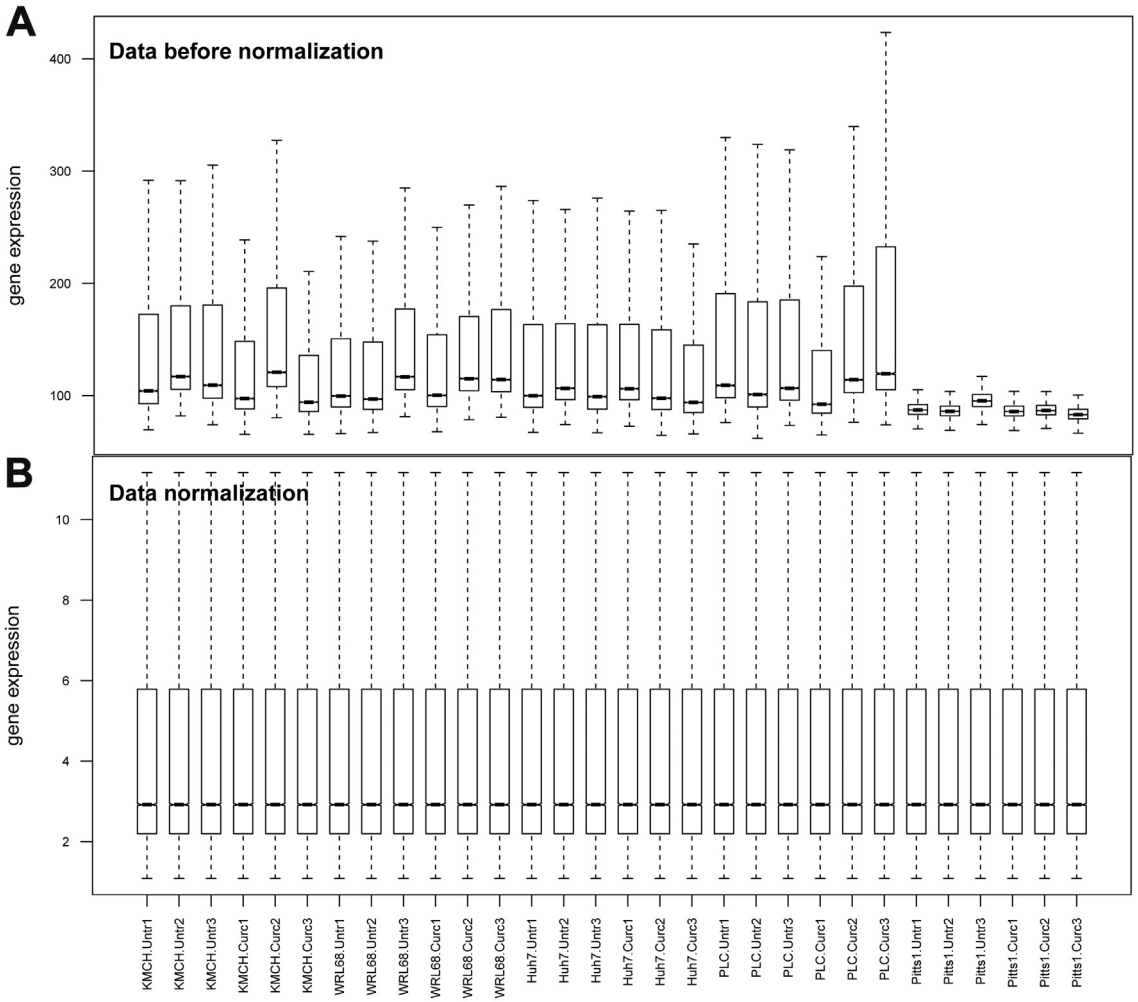

Fig. 1. Before (A) and after (B) data normalization. The x-axis presents the names of the samples, and the y-axis presents the distribution of gene expression values for each sample.

advent of next-generation sequencing, somatic mutations in genes such as *CTNNB1*, *TP53*, *AXIN1*, *TERT*, *ARID2*, *NFE2L2*, *KEAP1*, *ARID1A*, *CTNNB1*, *ARID1A*, and *AXIN1* have been confirmed or identified to be associated with HCC (Fujimoto et al., 2012; Kan et al., 2013; Kawai-Kitahata et al., 2016). Additionally, the involvement of signaling pathways including the RAS/MAPK and JAK/STAT pathways in HCC has been confirmed (Guichard et al., 2012). Additionally, a number of microRNAs (miRNAs) are transcriptionally upregulated or downregulated in HCC (Li et al., 2016). For example, miR-21 has been reported to be upregulated during HCC migration and invasion (Landgraf et al., 2007). miR-224 has been suggested to be upregulated throughout the development of HCC (Chen et al., 2012). miR-195 and miR-125b are downregulated in HCC (Xu et al., 2009; Liang et al., 2010). Although numerous genes and miRNAs associated with HCC have been identified, significant uncertainty remains because their molecular mechanisms have not been elucidated.

Curcumin, which is the principal polyphenolic curcuminoid that is also known as turmeric in Asia, is isolated from the plant *Curcuma longa* (Kossler et al., 2012). As a traditional herbal medicine, curcumin has long been used to treat several diseases and conditions, such as asthma, diabetes, abdominal pain, and renal fibrosis (Prasad and Aggarwal, 2011; Yadav and Aggarwal, 2011; Zhang et al., 2013; Sun et al., 2017).