RESEARCH ARTICLE

MiR-302 targets PAK5 and prevents the transition from chronic hepatitis B to liver cirrhosis

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Abstract

Objective: To determine and compare the levels of p21-activated kinase 5 messenger ribonucleic acid and micro-ribonucleic acid 302 in the serum of patients with chronic hepatitis B, liver cirrhosis and healthy individuals, and to analyse the roles and correlation of p21-activated kinase 5 messenger ribonucleic acid and micro-ribonucleic acid 302 in the progression of chronic hepatitis.

Method: The observational, clinical, case-control study was conducted at the Department of Laboratory Medicine, Affiliated Hospital of Hangzhou Normal University, Hangzhou, China, from February 2021 to January 2022. Peripheral blood serum samples were collected from healthy individuals undergoing physical examinations, as well as from patients with chronic hepatitis B and hepatitis B-related liver cirrhosis. Total ribonucleic acid was isolated and purified using the magnetic bead method, and the relative expression levels of micro-ribonucleic acid 302 and p21-activated kinase 5 messenger ribonucleic acid were determined using quantitative real-time polymerase chain reaction. Clinical test results were retrospectively analysed, and differences between the groups were assessed. Data was analysed using SPSS 22.

Results: Of the 70 participants, 18(25.71%) were healthy individuals with median age 33 years (interquartile range: 25-36.25 years), 18(25.71%) were patients with chronic hepatitis B having median age 34 years (interquartile range: 26-42 years), and 34(48.57%) were patients with cirrhosis having median age 54 years (interquartile range: 44-62 years). Overall, there were 50(71%) males and 20(29%) females with mean age 42.7±13.8 years. Median albumin (p=0.0365) and platelet (p=0.0116) levels were significantly lower in cirrhosis group compared to chronic hepatitis B group. Median aspartate aminotransferase level (p=0.0400) and aspartate aminotransferase/platelet ratio (p=0.0053) of chronic hepatitis B group were significantly higher than the healthy group (n=18). The expression of p21-activated kinase 5 was significantly increased in cirrhosis group compared to chronic hepatitis B group (p=0.0444) and healthy controls (p=0.0089). Compared to the healthy individuals, the expression of micro-ribonucleic acid 302 was significantly increased in both chronic hepatitis B (p=0.0237) and cirrhosis groups (p=0.0428).

Conclusions: P21-activated kinase 5 represented a promising therapeutic target for liver disease, and micro-ribonucleic acid 302 could serve as a therapeutic small molecule if an appropriate delivery system is available, particularly for liver fibrosis caused by hepatitis B virus infection.

Key Words: MicroRNA, miR-302, PAK5, HBV, Liver fibrosis, Cirrhosis.

(JPMA 74: 2114; 2024) **DOI: https://doi.org/10.47391/JPMA.11128**

Introduction

Liver cirrhosis is a common condition that affects people in low- and middle-income countries (LMICs), and is known to cause significant morbidity and mortality. Cirrhosis occurs as a result of long-term chronic liver inflammation, especially hepatitis B virus (HBV) infection, leading to widespread fibrosis in the liver.[1] With the help of vaccines, the number of HBV patients is decreasing year by year. However, for those who are already infected with HBV and have chronic hepatitis B (CHB), the vaccine is no longer effective. If the inflammation is not controlled, CHB is likely to progress to cirrhosis and even liver cancer.[2] Therefore, it is crucial to uncover the mechanisms behind the development of cirrhosis and to prevent its occurrence.

P21-activated kinases (PAKs) are a type of serine/threonine kinases that act as downstream effectors for Cell Division Cycle 42 (Cdc42) and Ras-related C3 botulinum toxin substrate (Rac), which are subfamilies of Rho small GTP hydrolases (GTPases). In mammals, PAKs (PAK1-6) are classified into group I (PAK1, PAK2, PAK3) and group II (PAK4, PAK5, PAK6) based on their structure

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Submission complete: 14-11-2023 **First Revision received:** 26-01-2024

Acceptance: 07-09-2024 **Last Revision received:** 05-09-2024

and sequence similarity. Previous studies have shown that PAK5 is often overexpressed in tumour progression, potentially due to its involvement in promoting cell activation, transformation, proliferation and inhibiting apoptosis.[3] In human hepatocellular carcinoma (HCC), for example, the messenger ribonucleic acid (mRNA) and protein of PAK5 are robustly upregulated in HCC tissues and cell lines, promoting hepatocytes proliferation and tumorigenicity.[4] High expression of PAK5 counteracts the inhibitory effects of micro-ribonucleic acid 129 (miR-129) on HCC cell transformation and proliferation.[5] PAK5 mRNA and protein level are markedly increased, and decreased miR-138-1-3p-induced HCC cell apoptosis.[6] HBV infection often leads to the development of liver cirrhosis, which can progress to HCC. It is currently unknown whether PAK5 gene expression starts to increase in the stage of liver cirrhosis and whether PAK5 plays a role in promoting the transformation of liver cirrhosis to HCC.

On their part, miRNAs are important regulators in gene post-transcription, degrading mRNA levels and reducing translation binding to the 3'-untranslated regions (UTRs) of the target gene. Recent studies have shown that miRNAs play a role in the development of liver fibrosis. Liver fibrosis is prevented by the upregulation of miR-34 through activating c-Jun N-terminal kinase (JNK) and Forkhead Box O3 (FOXO3).[7] Kupffer cells generate miR-690 internally and transport it to other liver cells through exosome secretion, which directly suppresses fibrogenesis in hepatic stellate cells.[8] Although there have been reports of the inhibitory effects of miR-302 on HCC progress,[9] it is still unclear whether miR-302 is involved in the development process of CHB to liver cirrhosis and HCC.

The current study was planned to determine and compare the levels of PAK5 mRNA and miR-302 in the serum of patients with CHB, liver cirrhosis and healthy individuals, and to analyse the roles and correlation of PAK5 mRNA and miR-302 in the progression of chronic hepatitis.

Materials and Methods

The observational, clinical, case-control study was conducted at the Department of Laboratory Medicine, Affiliated Hospital of Hangzhou Normal University, Hangzhou, China, from February 2021 to January 2022. After obtaining approval from the institutional ethics review committee, the sample was collected using convenience non-probability sampling technique, and written informed consent was taken from all the subjects.

The sample size was determined using an online calculator in 'Compare 2 Means: 2-Sample, 2-Sided Equality' mode. Type I error rate alpha (α) was set at 5%, Type II error beta (β) at 0.1, and power 1-β at 0.9. The ratio of PAK5/Actin beta (ACTB) and miR-302/ACTB in the healthy group and the diseased group was taken to range 1-10, with a mean of about 2-4 and a standard deviation (SD) of around 2[10]. To account for missing data and other potential factors, the sample size was inflated by 10%.

According to the diagnostic criteria for cirrhosis in the latest edition of the Guidelines for the Diagnosis and Treatment of Cirrhosis, formulated by the Hepatology Branch of the Chinese Medical Association (CMA), residual serum was collected from cirrhosis patients, CHB patients and healthy individuals. Patients with liver disease other than HBV, those who had undergone a liver transplant, or those who had HCC prior to enrolment were excluded.

A venous blood sample of 2ml was collected from the forearm elbow vein using a vacuum blood collection tube without anticoagulant. The samples were allowed to clot naturally at room temperature for 30 minutes, then centrifuged at 3000rpm for 5 minutes to collect the supernatant, which was the serum. After conducting routine medical tests, the serum was stored at -80°C temporarily.

Total RNA in 200µL serum were isolated with nucleic acid extraction and purification kits (S1006, Sansure, China) according to the manufacturer's protocol. Briefly, after adding lysing buffer to the serum, the mixture was vortexed and incubated in 95°C for 10 minutes. The total RNA released was captured by magnetic beads, which were mixed and left to rest at room temperature for 20 minutes. The sample tubes were fixed on a magnetic stand for 3 minutes, and the liquid was then discarded. The magnetic beads were washed twice with washing buffer, and the residual reagent was absorbed. The tubes were removed, and the beads were immersed in 30µL of ribonuclease (RNase)-free double distilled water for 10 minutes at room temperature. The tubes were then fixed on the magnetic stand again for another 3 minutes, and the RNA-containing liquid was transferred to a new RNase-free tube.

After elution, the total RNA was utilised for cDNA synthesis with the Mir-X miRNA First-Strand Synthesis Kit (638313, Takara, Japan) following the manufacturer's protocol. The quantitative real-time polymerase chain reaction (qRT-PCR) was performed using the SYBR-Green method as per the instructions of the user manual (639676, Takara, Japan). To determine the level of miR-302 and PAK5 mRNA, the delta-delta Ct method was

employed, with ACTB mRNA serving as the reference. The primers for PAK5 were as follows: forward 5′-AGTACATCTCCACGGCTTCCTACC-3′, and reverse 5′-GCTGCTGGTCGGAGGAGGAG-3′. The miR-302 specific 5′-primer was 5′-TAAGTGCTTCCATGTTTTGGTGA-3', with the 3′-primer included in the kit. And the primers for ACTB were as follows: forward 5′-GGCACCACACCTTCTACAATGAGC-3′, and reverse 5′-GATAGCACAGCCTGGATAGCAACG-3′. Bioinformatics analysis was done with TargetScan to identify potential miRNAs targeting PAK5.[11]

Data was analysed using SPSS 22. Samples with missing clinical data were excluded from statistical analysis. Shapiro Wilk test was used to determine data normality. The normally distributed parameters were reported as mean +/- standard deviation, while data not normally distributed was reported as median and interquartile range (IQR). The significance of miR-302 and PAK5 mRNA expression in different groups was evaluated using one-way analysis of variance (ANOVA) rank sum test and Dunnett's multiple comparison test. The correlation between miR-302 and PAK5 was determined using Spearman analysis, and statistical significance was assessed, with $p<0.05$ considered statistically significant. Mean ± standard error of mean (SEM) was used to express the data, and the charts were generated using GraphPad Prism 8.0 or Excel 2013.

Results

Of the 70 participants, 18(25.71%) were healthy individuals with median age 33 years (IQR: 25-36.25 years), 18(25.71%) were patients with chronic hepatitis B having median age 34 years (IQR: 26-42 years), and 34(48.57%) were patients with cirrhosis having median age 54 years (IQR: 44-62 years). Overall, there were 50(71%) males and 20(29%) females with mean age 42.7±13.8 years (Table 1).

Compared to CHB group, median ALB (p=0.0365) and PLT (p=0.0116) levels were significantly lower in the cirrhosis group. However, compared to the healthy control group, the CHB group did not show a significant decrease in ALB and PLT levels (Figure 1A-B). The level of aspartate aminotransferase (AST) (p=0.0400) and AST/PLT ratio (p=0.0053) increased significantly in the CHB group compared to the healthy control groups, but from the CHB stage to that of liver cirrhosis, the elevation of AST and AST/PLT ratio was not significant (Figure 1C-D).

Table-1: Baseline characteristics of clinical samples, according to the diagnosis at enrollment.

	Healthy （n=18）	CHB （n=18）	Cirrhosis （n=34）
Age [years, M (P25, P75)]	33.00 (25.00, 36.25)	34.00 (26.00, 42.00)	54.00 (44.00, 62.00)
ALB [g/L, M (P25, P75)]	46.45 (43.45, 48.35)	44.80 (43.78, 47.35)	39.35 (30.00, 44.53)
AST [U/L, 15.50 (14.00, 23.00) M (P25, P75)]		33.50 (21.50, 73.50)	28.50 (21.50, 46.00)
PLT [$\times 10^9$/L, M (P25, P75)]	229.00 (207.50, 254.25)	183.50 (144.00, 209.00)	85.50 (46.50, 150.00)
AST/PLT [M (P25, P75)]	0.07 (0.06, 0.09)	0.14 (0.04, 0.66)	0.37 (0.16, 0.82)
PT [s, M (P25, P75)]	—	11.90 (11.70, 13.90)	12.50 (11.40, 15.20)
INR [M (P25, P75)]	—	1.09 (1.08, 1.29)	1.16 (1.06, 1.42)

*Footnotes: CHB, chronic hepatitis B; ALB, albumin; AST, aspartate amino transferase; PLT, platelet; PT, prothrombin time; INR, international normalized ratio.

The expression of PAK5 was significantly increased in the cirrhosis group compared to the CHB group (p=0.0444) and healthy controls (p=0.0089) (Figure 2A). And the PAK5 level decreased in 7(41.2%) CHB samples, but it

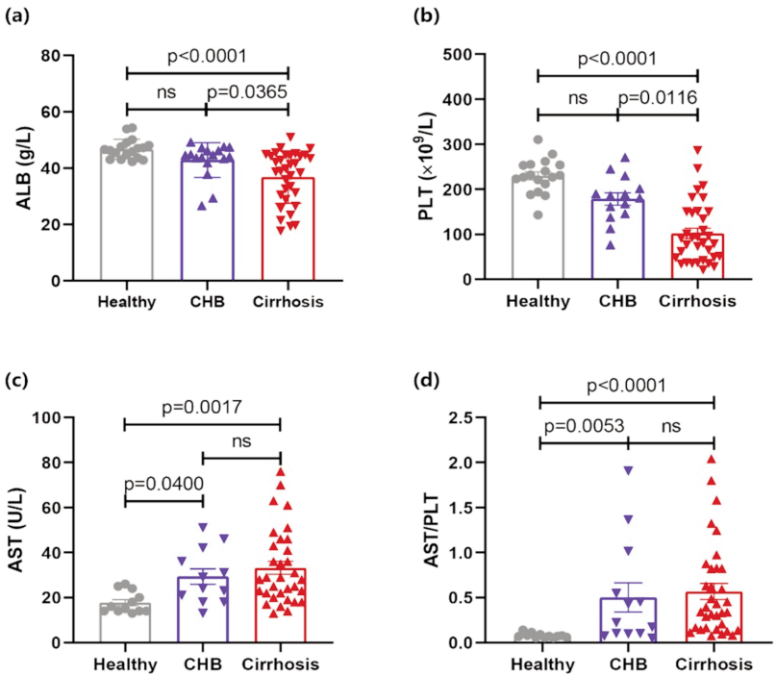

Figure-1: Clinical test data of experimental samples related to healthy control group, chronic hepatitis B (CHB) group, and cirrhosis group for (A) albumin, (B) platelets (PLT), (C) aspartate aminotransferase (AST), and (D) the ratio of AST to PLT (AST/PLT), intergroup comparisons were performed using rank sum test and Dunnett's multiple comparison test ($p<0.05$).

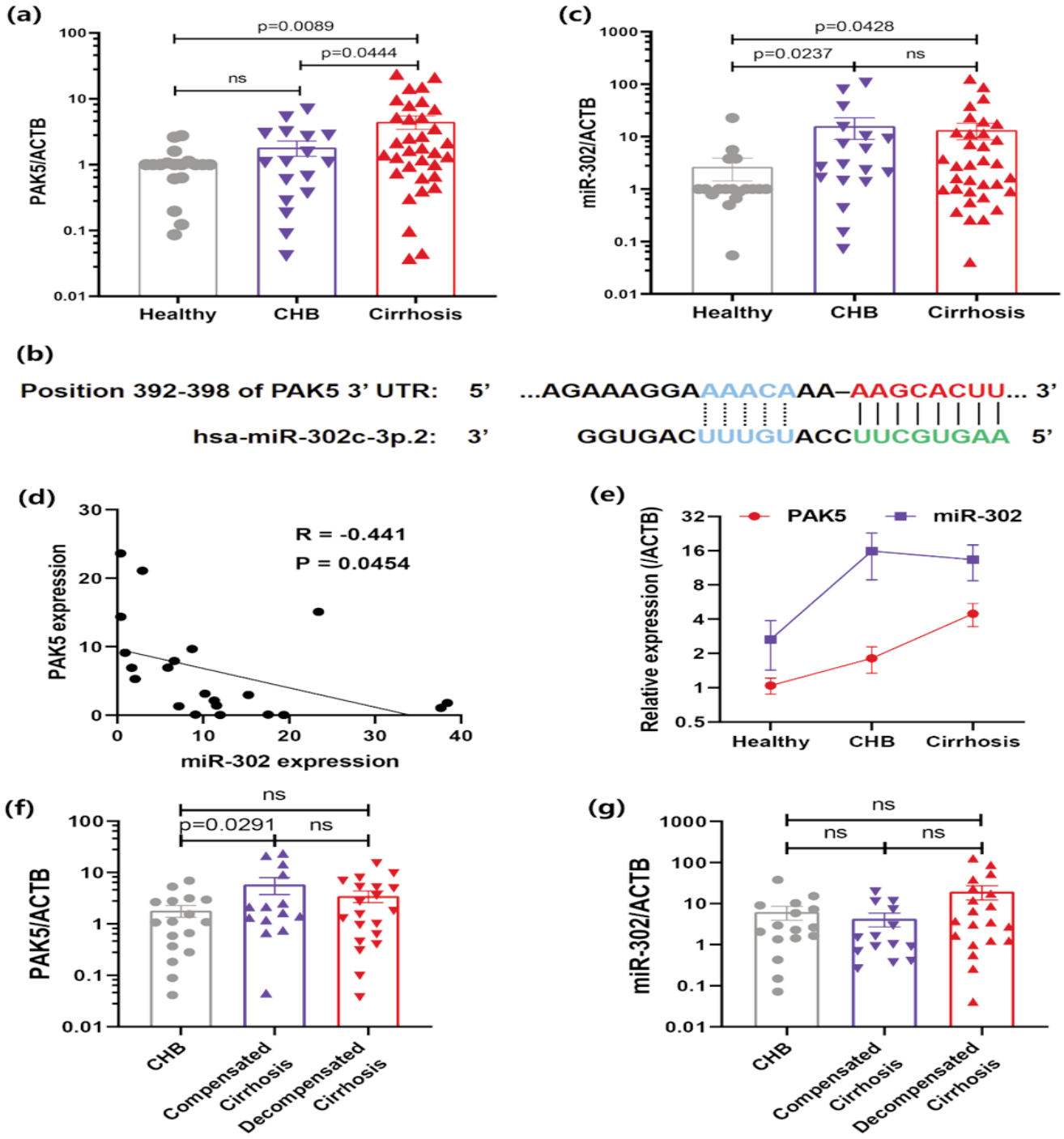

Figure-2: Micro-ribonucleic acid 302 (miR-302) targeted and inhibited the expression of p21-aactivated kinase 5 (PAK5) gene and was negatively correlated with PAK5. (A) By using quantitative real-time polymerase chain reaction (qRT-PCR) to detect the content of PAK5 in three groups of serum samples, actin beta (ACTB) was used as an internal reference to normalise the relative expression level of PAK5. Each sample was tested in triplicate. (B) Using TargetScan to predict the relevant parameters of micro-ribonucleic acid 302 (miR-302) targetting binding to PAK5 3'untranslated region (UTR). Putative binding site for miR-302 in the 3'UTR of PAK5 was displayed. (D) The content of miR-302 in the three groups was detected using qRT-PCR. ACTB was utilised as an internal reference to normalise the relative expression level of miR-302. Each sample was tested in triplicate. (D) The relevance of miR-302 and PAK5 and the statistical significance were assessed using spearman analysis. (E) The changing trends in the expression of miR-302 and PAK5 genes in the course of chronic hepatitis B (CHB). (F and G) Compared to CHB, the relative expression of PAK5 the miR-302 in the compensated and decompensated stages of liver cirrhosis. ACTB was used as an internal reference. Each sample was tested in triplicate.

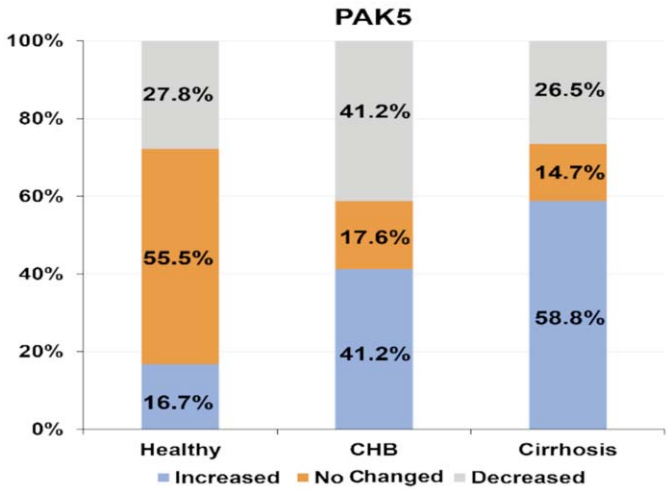

Figure-3: Changes in PAK5 content in other serum samples relative to the test result of a healthy individual's serum. The value 100% represents the total proportion of samples in each group.

increased in 20(58.8%) cirrhosis samples (Figure 3).

Further, miR-302 was one of the potential regulators of PAK5, and this interaction occurred through binding to the 3'UTR of PAK5, with 8 matching positions between mature miR302 and PAK5 mRNA. Additionally, there were 5 potential binding sites (AAACA) in close proximity to the seed sequence (AAGCACUU) (Figure 2B). The context++ score, which measured the strength of the pairing between miR-302 and PAK5, also known as PAK7, was -0.35, the percentile of the context++ score was as high as 98, indicating that PAK5 was likely the true target of miR-302 (Figure 4).

The expression of miR-302 was significantly increased in both CHB (p=0.0237) and cirrhosis (p=0.0428) groups compared to the healthy individuals. However, in the case of cirrhosis, the expression of miR-302 showed a slight decrease, although it was not very evident (Figure 2C). Further analysis of the data indicated that miR-302 was elevated in 15(83.3%) CHB samples, whereas it was only elevated in 18(52.9%) cirrhosis samples. Conversely, the proportion of decreased or unchanged miR-302 expression was noted in 3(16.7%) CHB samples compared to 16(47.1%) in the cirrhosis group (Figure 5).

Among the CHB and cirrhosis samples, 21(40.4%) with significantly elevated PAK5, and when miR-302 increased, PAK5 decreased, and, conversely, when PAK5 increased, miR-302 decreased (Figure 6). High expression of miR-302 was associated with low expression of PAK5 and vice

	Predicted consequential pairing of target region (top) and miRNA (bottom)	Site type	Context++ score	Context++ score percentile	Weighted context++ score	Conserved branch length	P_{CT}	Predicted relative K_D
Position 392-398 of PAK7 3' UTR	5' ...AGAAAGGAAAACAAA-AAGCACUU...	7mer-m8	-0.35	98	-0.35	4.514	0.70	-4.790
hsa-miR-302c-3p.2	3' GGUGACUUUUGUACCUUCGUGAA							

Figure-4: Predicting the relevant parameters of miR-302 targeting binding to PAK5 3'UTR.
miR-302: Micro-ribonucleic acid 302, PAK5: P21-aactivated kinase 5, UTR: Untranslated region.

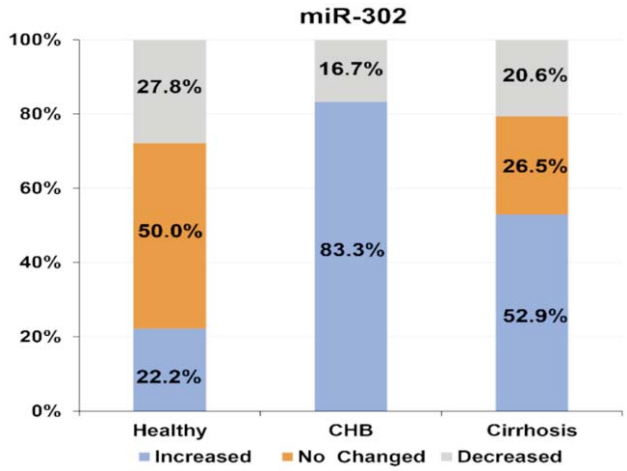

Figure-5: Relative changes in the content of miR-302 in other serum samples based on the test result of one healthy individual's serum. The value 100% represents the total proportion of samples in each group.
miR-302: Micro-ribonucleic acid 302.

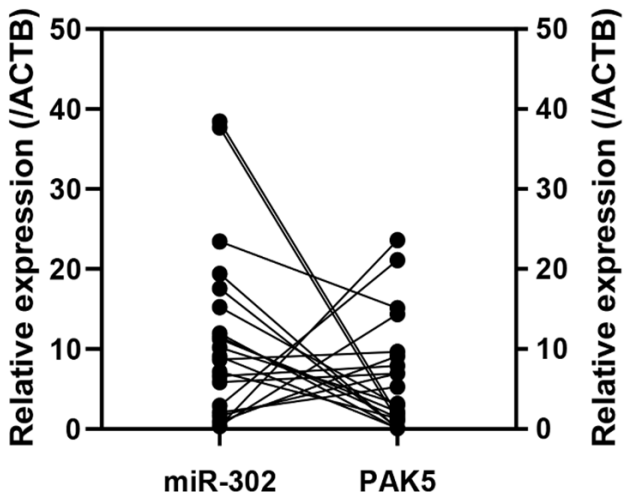

Figure-6: The correlation between the levels of miR-302 and PAK5 in the samples with increased concentrations.
PAK5: P21-aactivated kinase 5, miR-301: Micro-ribonucleic acid 302, ACTB: Actin beta.

versa, indicating a significant negative correlation between PAK5 and miR-302 (r=-0.441, p=0.0454) (Figure 2D).

MiR-302 appeared to have a role in preventing further damage to liver cells caused by HBV, and as the expression of the detrimental factor PAK5 gradually increased, the expression of miR-302 started to decline, with its hepatoprotective effect weakening accordingly (Figure 2E).

The liver cirrhosis samples were further divided into two groups: compensated and decompensated. The expression of PAK5 in the compensated subgroup significantly increased (p=0.0291) compared to CHB, while there were no significant difference between the other subgroup and CHB (Figure 2F-G).

Discussion

The current study demonstrated an inverse correlation between miR-302 and PAK5. Therefore, boosting miR-302 expression may serve as a means to preventing or alleviating the heightened expression of PAK5. Nonetheless, chemical therapy faces significant challenges in treating HBV infection. Therefore, the current study of the miR-302-PAK5 axis presents promising opportunities for developing new strategies in this context.

Liver cirrhosis is widely prevalent in the world and is associated with high morbidity and mortality. There are about 1 million deaths globally each year caused by viral hepatitis and HCC, and another 1 million deaths are annually caused by cirrhosis.[12] Cirrhosis is a consequence of chronic liver inflammation that is followed by diffuse hepatic fibrosis, wherein the normal hepatic architecture is replaced by regenerative hepatic nodules, and evolves from an asymptomatic phase (compensated cirrhosis) to a symptomatic phase (decompensated cirrhosis), which eventually leads to liver failure and death. The compensated stage has no obvious clinical symptoms, while the decompensated stage is characterised by portal hypertension and severe liver dysfunction, and the liver's ability to synthesise proteins, such as ALB and clotting factors, is reduced, leading to malnutrition and a tendency to bleed in patients.[13]

Liver tissue biopsy is considered the most reliable method for diagnosing and assessing the early stages of liver fibrosis and inflammation caused by various factors. However, liquid biopsy has become increasingly popular for disease monitoring due to its convenience and effectiveness.[14] In clinical practice, traditional serological testing is essentially a part of liquid biopsy, with targets often being free proteins, lipids, polysaccharides, or inorganic substances in the serum. The current study inferred that the expression of PAK5 was suppressed by miR-302, and miR-302 had a certain protective effect on the damaged liver.

PAK5 is overexpressed in liver cancer cells, playing a role in promoting cancer. To observe whether PAK5 had already begun to be overexpressed in the hepatitis and cirrhosis stages, the current study detected the level of PAK5 mRNA in the serum. The results showed that PAK5 expression tended to increase, but there were no significant changes between healthy controls and CHB. Furthermore, the results suggested that HBV infection led to increased levels of miR-302, and the reduction in cirrhosis may be attributed to alternative mechanisms. It is plausible that the overexpression of miR-302 could hinder the development of hepatocyte fibrosis, as it initially increased during CHB and subsequently decreased during the cirrhosis stage, which marked the progression of HBV infection.

Numerous studies have shown that miRNA expression is disrupted in various liver diseases, including hepatitis B and C viral infections, liver cirrhosis and cancer.[15-18] MiR-302 is expressed at low levels in HCC cells, and overexpressed miR-302 function is a potential suppressor of tumour angiogenesis targetting metastasis-associated in colon cancer 1 (MACC1)[9] and suppresses cell proliferation by targeting epidermal growth factor receptor (EGFR).[19] In recent years, miR-302 has been reported to be regulated in various infections, including enterovirus-71,[20] influenza A virus,[21] and human immunodeficiency virus (HIV).[22] However, the expression of miR-302 in HBV infection has not been determined yet, the specific impact of miR-302 on HBV infection and liver fibrosis progression has not been thoroughly investigated, and whether miR-302 can directly bind to the 3'UTR region of PAK5 mRNA as predicted, leading to the degradation of PAK5 mRNA and blocking its translation, still needs to be confirmed through experimental validation.

The current study indicated that miR-302 was commonly elevated in individuals with HBV infection and played a role in controlling the development of liver fibrosis and progression to cirrhosis. Additional tests showed that PAK5 was a potential target of miR-302 and that the decrease in PAK5 was partly responsible for the liver fibrosis functions of miR-302. Therefore, it may be worthwhile to explore the restoration of miR-302 and inhibition of PAK5 as potential therapeutic approaches, as well as identifying additional targets for the development of clinical drugs. The current study identified that PAK5

not only promoted cancer, but also promoted the occurrence of cirrhosis, and miR-302 could inhibit the progression of chronic liver disease. However, the specific mechanisms still need further investigation.

The current study has limitations as the findings are based on a small sample size which was not enough to explore some pathological phenomena. There were more females 11(61%) in the healthy group compared to males 7(39%), while there were more males 14(78%) in the CHB group compared to females 4(22%) and in the cirrhosis group 29(85.3%) compared to 5(14.7%). This may have affected the findings.

Despite no significant differences in the compensated and decompensated liver cirrhosis data, a negative correlation was observed between miR-302 and PAK5, possibly due to the small sample size and considerable experimental errors. So future studies with large sample sizes and in-depth research on the mechanisms are needed to validate the findings.

PAK5 is expected to become a potential target for the treatment of chronic liver disease, and the combination of miR-302 with advanced nanomedicine technology also holds promise as a targeted therapy, since the coronavirus disease-2019 (COVID-19) mRNA vaccine encapsulated in nanoliposomes has been successfully applied in clinical practice.[23]

Conclusion

There was an inverse relationship between miR-302 and PAK5, with high miR-302 linked to low PAK5 expression in healthy individuals as well as CHB and liver cirrhosis patients, indicating that miR-302 may protect liver cells from HBV damage, while PAK5 expression may weaken this protective effect.

Acknowledgments: We are grateful to Dr Chengsong Cai and Jiahuan Chen for technical assistance with qRT-PCR, and to Dr Wangqi Yu and Gaoming Zheng for assistance with clinical experiments, helpful discussions and suggestions.

Disclaimer: None.

Conflict of Interest: None.

Source of Funding: The Natural Science Foundation of Zhejiang Province **(LGF20H200007)**, and Hangzhou Science and Technology Development Plans **(20201231Y026, 20110833B29)**, China.

References

1. Sepanlou SG, Safiri S, Bisignano C, Ikuta KS, Merat S, Saberifiroozi M, et al. The global, regional, and national burden of cirrhosis by cause in 195 countries and territories, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. Lancet Gastroenterol Hepatol. 2020; 5:245-66. doi: 10.1016/S2468-1253(19)30349-8
2. Hsu YC, Huang DQ, Nguyen MH. Global burden of hepatitis B virus: current status, missed opportunities and a call for action. Nat Rev Gastroenterol Hepatol. 2023; 20:524-37. doi: 10.1038/s41575-023-00760-9.
3. Li YK, Zou J, Ye DM, Zeng Y, Chen CY, Luo GF, et al. Human p21-activated kinase 5 (PAK5) expression and potential mechanisms in relevant cancers: Basic and clinical perspectives for molecular cancer therapeutics. Life Sci. 2020; 241:117113. doi: 10.1016/j.lfs.2019.117113.
4. Fang ZP, Jiang BG, Gu XF, Zhao B, Ge RL, Zhang FB. P21-activated kinase 5 plays essential roles in the proliferation and tumorigenicity of human hepatocellular carcinoma. Acta Pharmacol Sin. 2014; 35:82-8. doi: 10.1038/aps.2013.31.
5. Zhai J, Qu S, Li X, Zhong J, Chen X, Qu Z, et al. miR-129 suppresses tumor cell growth and invasion by targeting PAK5 in hepatocellular carcinoma. Biochem Biophys Res Commun. 2015; 464:161-7. doi: 10.1016/j.bbrc.2015.06.108.
6. Li TT, Mou J, Pan YJ, Huo FC, Du WQ, Liang J, et al. MicroRNA-138-1-3p sensitizes sorafenib to hepatocellular carcinoma by targeting PAK5 mediated β-catenin/ABCB1 signaling pathway. J Biomed Sci. 2021; 28:56. doi: 10.1186/s12929-021-00752-4.
7. Piccolo P, Ferriero R, Barbato A, Attanasio S, Monti M, Perna C, et al. Up-regulation of miR-34b/c by JNK and FOXO3 protects from liver fibrosis. Proc Natl Acad Sci U S A. 2021; 118:e2025242118. doi: 10.1073/pnas.2025242118.
8. Gao H, Jin Z, Bandyopadhyay G, Rocha KCE, Liu X, Zhao H, et al. MiR-690 treatment causes decreased fibrosis and steatosis and restores specific Kupffer cell functions in NASH. Cell Metab. 2022; 34:978-90.e4. doi: 10.1016/j.cmet.2022.05.008.
9. Cao YP, Pan M, Song YL, Zhang HL, Sui HT, Shan BC, et al. MiR-302a/b/c suppresses tumor angiogenesis in hepatocellular carcinoma by targeting MACC1. Eur Rev Med Pharmacol Sci. 2019; 23:7863-73. doi: 10.26355/eurrev_201909_18996.
10. Ahmad A, Mustafa G, Mazari N, Naveed MA. Comparison of thrombomodulin, vWF, and ADAMTS13 levels between preeclampsia and normal pregnancy. J Pak Med Assoc. 2024; 74:38-42. doi: 10.47391/JPMA.7537.
11. Agarwal V, Bell GW, Nam J, Bartel DP. Predicting effective microRNA target sites in mammalian mRNAs. eLife. 2015; 4:e05005. doi: 10.7554/eLife.05005.
12. Ginès P, Krag A, Abraldes JG, Solà E, Fabrellas N, Kamath PS. Liver cirrhosis. Lancet. 2021; 398:1359-76. doi: 10.1016/S0140-6736(21)01374-X.
13. Arroyo V, Moreau R, Jalan R. Acute-on-Chronic Liver Failure. N Engl J Med. 2020; 382:2137-45. doi: 10.1056/NEJMra1914900.
14 Feng T, Lai C, Yuan Q, Yang W, Yao Y, Du M, et al. Non-invasive assessment of liver fibrosis by serum metabolites in non-human primates and human patients. iScience. 2023; 26:107538. doi: 10.1016/j.isci.2023.107538.
15. Tadokoro T, Morishita A, Masaki T. Diagnosis and Therapeutic Management of Liver Fibrosis by MicroRNA. Int J Mol Sci. 2021; 22:8139. doi: 10.3390/ijms22158139.
16. Wang W, Liu R, Su Y, Li H, Xie W, Ning B. MicroRNA-21-5p mediates TGF-beta-regulated fibrogenic activation of spinal fibroblasts and the formation of fibrotic scars after spinal cord injury. Int J Biol Sci. 2018; 14:178–88. doi: 10.7150/ijbs.24074.
17. Shrivastava S, Petrone J, Steele R, Lauer GM, Bisceglie AMD, Ray RB. Up-regulation of circulating miR-20a is correlated with hepatitis C virus-mediated liver disease progression. Hepatology. 2013; 58:863–71. doi: 10.1002/hep.26296.
18. Roderburg C, Urban GW, Bettermann K, Vucur M, Zimmermann H,

Schmidt S, et al. Micro-RNA profiling reveals a role for miR-29 in human and murine liver fibrosis. Hepatology. 2011; 53:209–18. doi: 10.1002/hep.23922.

19. Wang L, Yao J, Shi X, Hu L, Li Z, Song T, et al. MicroRNA-302b suppresses cell proliferation by targeting EGFR in human hepatocellular carcinoma SMMC-7721 cells. BMC Cancer. 2013; 13:448. doi: 10.1186/1471-2407-13-448.
20. Peng N, Yang X, Zhu C, Zhou L, Yu H, Li M, et al. MicroRNA-302 Cluster Downregulates Enterovirus 71-Induced Innate Immune Response by Targeting KPNA2. J Immunol. 2018; 201:145-56. doi: 10.4049/jimmunol.1701692.
21 Chen X, Zhou L, Peng N, Yu H, Li M, Cao Z, et al. MicroRNA-302a suppresses influenza A virus-stimulated interferon regulatory factor-5 expression and cytokine storm induction. J Biol Chem. 2017; 292:21291-303. doi: 10.1074/jbc.M117.805937.
22. Lin W, Li XF, Ren DC, Song M, Duan L, Liu JZ, et al. Administration of zoledronic acid alleviates osteoporosis in HIV patients by suppressing osteoclastogenesis via regulating RANKL expression. Mol Med. 2021; 27:19. doi: 10.1186/s10020-021-00276-5.
23. Suzuki Y, Ishihara H. Difference in the lipid nanoparticle technology employed in three approved siRNA (Patisiran) and mRNA (COVID-19 vaccine) drugs. Drug Metab Pharmacokinet. 2021; 41:100424. doi: 10.1016/j.dmpk.2021.100424.

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YZ, XL, LW: Data collection, literature search, experimental work, analysis and critical revision.
XP, QK: Study Design, literature search, drafting, data analysis, interpretations, writing, proofreading, supervision and final approval.
FP: Study Design, literature search, drafting, supervision and final approval.