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Preliminary screening and functional analysis of circular RNAs associated with hepatic stellate cell activation ¹

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Abstract: Objective: To screen for circular RNAs (circRNAs) that are associated with the activation of hepatic stellate cell (HSC) by monitoring changes in liver circRNA expression in a model of liver fibrosis. **Methods:** The classic mouse model of CCl₄-induced liver fibrosis was established and validated by histopathological examination. JS1 cells were activated by TGF- β 1 to model HSC activation *in vitro*. Differentially expressed circRNAs in the fibrotic liver tissues and JS1 cells were determined using circRNA microarray, and some of those circRNAs were verified by RT-qPCR. The target genes of the above circRNAs were then predicted by bioinformatics analysis and summarized into a “circRNA-miRNA-mRNA” network diagram. Constructed plasmid mmu_circ_34116 siRNA was transfected to JS1 cells by Lipo2000, then we detected the expression changes of α -SMA. **Results:** A total of 10,389 circRNAs were identified by microarray screening, and 69 differentially expressed circRNAs were detected in the fibrotic liver tissues with more than 2-fold difference in expression level relative to normal liver tissues ($P < 0.05$); 14 circRNAs were up-regulated and 55 were down-regulated. Five differentially expressed circRNAs in fibrotic liver and JS1 cells were verified by RT-qPCR, while all five showed similar trends with the microarray results in the liver, only 3 circRNAs in the JS1 activation model were consistent with the microarray results while one showed no significant change and one circRNA was not detected. Bioinformatics analysis predicted that the “mmu_circ_34116/miR-22-3P/BMP7” signal axis might be involved in the activation of HSC. Transfection experiment confirmed that the expression of α -SMA is significantly elevated as a result of inhibitory expression of mmu_circ_34116. **Conclusion:** The circRNAs

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