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Exosomal circ-RBM23 sponging miR-139-5p to promote liver regeneration through RRM2/AKT/mTOR pathway

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Abstract

Background Exosomes are small nano-size membrane vesicles and are involved in intercellular interaction. Here, we examined if exosomes obtained from human placental stem cells promote liver regeneration after partial hepatectomy. **Methods** Exosomes generated from primary human placental stem cells were isolated and characterized. Cell co-culture model was used to clarify whether exosomes can induce hepatocytes proliferation *in vitro*. Partial hepatectomy mouse model was used to evaluate whether exosomes can promote hepatocytes proliferation *in vivo*. **Results** It is found that human placental-derived stem cells exosomes (hPDSCs-exo) can induce hepatocyte proliferation *in vitro* and *in vivo*. Mechanistically, exosomal circ-RBM23 served as a ceRNA for miR-139-5p, regulated RRM2 and accelerated proliferation through AKT/mTOR pathways. Ablation of exosomal circ-RBM23 suppressed the proliferative effect of exosomes. **Conclusions** The hPMSCs exosomal circ-RBM23 stimulated cell proliferation and liver regeneration after 70% partial hepatectomy by regulated RRM2. Our findings highlight a potential novel therapeutic avenue for liver regeneration after hepatectomy.

Keywords: Exosomes, circ-RNA23, placental stem cells, liver regeneration

1. Introduction

Liver regeneration refers to a compensatory process that replaces functional liver mass lost to injury or disease (1, 2). The mechanisms of this process are highly complex and have been researched for decades (3). Liver regeneration after partial hepatectomy (PH) is complex and well-orchestrated, implicating multiple factors including cytokines, exosomes, growth factors, and signaling pathways (2-5). Unravelling the mechanism and regulation of liver regeneration significantly helps liver resection patients.

Human placental mesenchymal stem cells (hPMSCs) exhibit self-renewing potential and differentiate into multiple cell type lineages (6). They secrete soluble factors with paracrine effects. The therapeutic potential of hPMSCs has been showed in tissue damage repair after ischemic diseases including stroke, and myocardial and cerebral infarctions (7-11). These studies suggest that hPMSCs-based therapies might be developed for future clinical applications.

Exosomes are membrane nano vesicles (30-100 nm) secreted by cells into the extracellular environment (12). They carry complex cargo loads, including proteins and RNAs that potentially affect cellular processes (13, 14). Circular RNAs (circ-RNAs) constitute a novel class of widespread, numerous transcripts that form a covalently closed continuous loop (15, 16). Recent studies demonstrated that circ-RNAs serve as efficient miRNA sponges and regulate liver regeneration (17-19). However, exosomal circ-RNAs together with their function in liver regeneration are not completely understood.

In this study, we investigated the effects of human placental-derived stem cells exosomes (hPDSCs-exo) on murine/human hepatocytes (*in vitro*) and a 70% partial hepatectomy model (*in vivo*), as well as the underlying mechanisms.

2. Materials and methods

2.1. Human placenta stem cell preparation

We previously described the methods for preparing hPMSCs (20). The collection and use of the samples were approved by the review board and ethics committee of Zhu Jiang Hospital (approval number: 2019-KY-015-02). All of the participants provided written informed consent prior to the sample collection.

2.2. RNase R treatment

RNase R (Epicentre Technologies) was utilized to degrade linear RNA. Total RNA (2µg) was incubated for 30 min at 37°C with zero units or 20 units of RNase R. Subsequently, RNA was purified by phenol-chloroform extraction, retro-transcribed, and used for RT-qPCR via Actin as reference gene.

2.3. Exosomes isolation

For exosomes extraction from the conditioned medium of cell cultures, 5×10^7 cells were seeded in 25 mL media supplemented with 10% exo-depleted FBS in a 150cm² tissue culture plate for 48 h. Standard differential centrifugation was used to isolate exosomes from the conditioned medium. The conditioned medium was subjected to sequential centrifugation steps of 200 g for 10 min and 2000 g for 15 min to eliminate many cells and cellular debris. Then, the supernatant was centrifuged for 10,000 g for 30min and filtered using a 0.22 µm filter (Millipore) to eliminate cell

debris membranes and EVs. A pellet was collected after 2 h of ultracentrifugation at 100,000 g using a P28Ti fixed angle rotor (Himac CP100xn, Japan) and resuspended in PBS. The resulting pellet was collected through ultracentrifugation for 70 min at 100,000 g and resuspended in PBS for downstream analyses.

2.4.Exosomes characterization

Exosomes were examined under transmission electron microscopy (H-600 HITACHI microscope, Japan) at 80 keV. Exosomes size and number were established and the data analyzed were using a Nanoparticle tracking analysis(NTA) ZetaView (Particle Metrix, GERMANY). Purity was examined by western blotting for exosome markers (CD9, CD63, CD81 and HSP70). The number of exosomes was examined by NTA.

2.5.High-throughput sequencing

High-throughput sequencing and OE Biotech (Shanghai, China) were used to determine the CircRNA expression profiles. Total RNA was extracted and determined, then a cDNA library constructed after removing ribosomal RNA and RNase R digestion. Illumina sequencer (HiSeq™ 2500) was utilized after passing quality inspection. Eventually, circRNAs were confirmed and statistically analyzed.

2.6.Bioinformatics analysis

Data Source: Gene expression profiles were obtained from GEO database (<https://www.ncbi.nlm.nih.gov/geo/>). Identification of DEGs and Heatmap and clustering analysis: Data were analyzed using R software (version 3.6.2) and its package, limma. $|\log FC| > 1$ and $p < 0.05$ were set as cut-off for identifying DEGs.

Heatmaps and clustering were generated using the R software and limma package.

Enrichment analysis of DEGs: The R software (version 3.6.2) was applied for functional and pathway enrichment analyses of the DEGs. The enrichment content included GO and KEGG pathways. Pathway enrichment analysis depended on references from KEGG pathways. False discovery rate (FDR) <0.05 was applied as cut-off.

2.7. Animals

Male C57BL/6 mice (6-8 weeks) were purchased from the animal center of Guangdong medical laboratory (Certificate of Conformity: SYXK (YUE) 2019-0215). The institutional animal care and use committee center of Zhu Jiang Hospital, Southern Medical University, Guangzhou, China approved the experimental protocols. The mice were housed in plastic cages at a controlled temperature of $22\pm 1^{\circ}\text{C}$. Standard rodent chow and water were provided. All animals and samples were assigned a number that did not reveal treatment group for blind analysis.

2.8. Murine 70% partial hepatectomy model

Anaesthetization was done using 70 mg/kg pentobarbital and two-thirds hepatectomy performed as described by Mitchell and Willenbring (21). The left, median, and posterior right lobes were ligated and excised, removing 65-70% (2/3 partial hepatectomy) of the liver.

2.9. RNA-binding protein immunoprecipitation (RIP) assay

Immunoprecipitation of the circ-RBM23 bound to Ago2 was done via a Magna

RIP™ RNA-binding protein immunoprecipitation kit (Merck Millipore, Germany).

2×10^7 L02 cells were harvested in RIP lysis buffer and lysates stored at -80°C . 8 μg of anti-Ago2 (MA5-23515, Invitrogen, USA) or normal control IgG was incubated with magnetic beads for 2 h at RT and 100 μL of the RIP lysate mixed with 900 μL of RIP immunoprecipitation buffer. This was then added to bead-antibody complexes and incubated overnight at 4°C . The beads were then mixed with proteinase K buffer and incubated for 30 min at 55°C and RNA extracted for PCR.

2.10. Dual-luciferase report assay

Dual-luciferase assay was conducted using a dual-luciferase reporter assay (Promega, Madison, WI, USA) as usual (22). L02 cells were seeded on 24-well plates and cultured to 50% confluence. Then, they were transfected with circ-RBM23-wt or circ-RBM23-mut plasmid and negative control renilla luciferase plasmid together with miR-139-5p for 48 h. Cells were lysed then luciferase and renilla substrates were added. This was followed by luciferase analysis after 24 h using a dual-luciferase reporter (Promega) based on the manufacturer's instructions.

2.11. Cell culture, exosomes labeling, cell transfection, flow cytometry, liver histological and serum analysis, western-blot, cell proliferation assays

Details are outlined in the supplemental materials.

2.12. Statistical analysis

Continuous variables are presented as mean $\pm$ standard error of the mean (SEM). Analysis of variance (ANOVA) and post hoc Bonferroni analysis was conducted for multiple comparisons using GraphPad Prism 8.0. $P < 0.05$ was considered statistically

significant.

3. Results

3.1 Identification of hPMSCs and hPDSCs-exo

The hPMSCs have the essential characteristics of MSCs and are positive for the surface cell marker CD38 and negative for CD34, CD45, and CD133 (Fig 1A). Exosomes were isolated from hPMSCs supernatant as described previously (12). Transmission electron microscopy (TEM) revealed that hPDSCs-exo had a round, ball-like shape (Fig 1B). NanoSight analysis (NTA) showed that hPDSCs-exo had diameters of 30-150 nm and the quantification of exosomes was showed in the right panels (Fig1C). Western blot analysis demonstrated that the collected hPDSCs-exo expressed precise exosomal surface markers including CD9, CD63, CD81, and HSP70 (Fig 1D). These results suggest that hPDSCs-exo exhibited specific exosomes characteristics that are identical to those described previously (12, 13).

3.2 hPDSCs-exo promoted liver regeneration *in vivo* and hepatocyte proliferation *in vitro*

To determine whether hPDSCs-exo induced hepatocyte regeneration *in vivo*, we administered hPDSCs-exo by intraperitoneal injection before 70% partial hepatectomy (PH) in mice. Relative to PH-only mice, exosomes treatment significantly increased liver mitosis 2 days after PH as revealed by H&E staining (Fig 2A-B). Additionally, PCNA IHC staining revealed that exosomes promoted cell proliferation in mice after PH (Fig 2C-D). Furthermore, we showed that relative to exosomes treated mice, untreated mice exhibited markedly higher levels of ALT and

AST, suggested greater liver injury (Fig 2E). To determine whether hPDSCs-exo affected hepatocyte proliferation, we treated cultured AML-12 and L02 cells with hPDSCs-exo which labeled with PKH26 (a cell membrane marker). Confocal analysis found that exosomes were directly taken up by the cells (Fig 2F). Moreover, CCK8 assays showed elevated cell proliferation after hPDSCs-exo treatment for 24 h (Fig 2G). These results indicating that hPDSCs-exo plays a proliferative and protective role in PH mice.

3.3 The identification and characteristics of circ-RBM23 in hPDSCs-exo

We profiled circular RNA expression in hPDSCs-exo using high-throughput sequencing. We first selected 14 possible circular RNAs based on their expression (Fig 3A) and found that circ-RBM23, circ-SWAP70, circ-MAPK9, circ-FKBP8, and circ-EPHB4 were highly expressed in hPDSCs-exo. RT-qPCR revealed that circ-RBM23 had the highest expression (Fig 3B, primer of circ-RNAs were showed in supplementary Table 1), hence it was therefore selected for subsequent analysis. Then we found that relative to linear RBM23, circ-RBM23 was resistant to RNase R digestion (Fig 3C). Primer of linear RBM23 and circ-RBM23 were showed in supplementary Table 2. Furthermore, Sanger sequencing and circPrimer2.0 were used to confirm the head-to-tail splicing, circular features and characterized circ-RBM23 expression in hPMSCs (Fig 3D). Next, we knocked down circ-RBM23 using siRNA in L02 cells and confirmed the silencing result using RT-qPCR ($p < 0.01$, Fig 3E). CCK8 assays also showed that downregulating circ-RBM23 blocked the proliferation of L02 cells (Fig 3F).

3.4 *Circ-RBM23 was served as a sponge for miR-139-5p*

Previous studies showed that circular RNA mainly functions as miRNA sponges. Then we made a prediction with circ-RBM23. The top-5 potential circ-RBM23 target miRNAs were predicted via a bioinformatics tool starBase2.0 (Primer of miRNAs was showed in supplementary Table 3). RT-qPCR analysis showed that circ-RBM23 silencing increased miRNA expression, and miR-139-5p was most significantly upregulated (Fig 3G). Dual-luciferase reporter analysis revealed that co-transfection of wild circ-RBM23 and miR-139-5p hindered the expression of Rluc, indicating the sponge effect of circ-RBM23 (Fig 3H). RNA immunoprecipitation (RIP) showed that circ-RBM23 was enriched in Ago2-containing immunoprecipitates than actin or miR-139-5p (Fig 3I), indicating that circ-RBM23 molecularly sponges miR-139-5p.

3.5 *Circ-RBM23 regulated miR-139-5p/RRM2 in cell proliferation*

In order to identify the important genes in liver regeneration, we used R software as usual to analyze dataset GSE97429 from GEO for genes involved in liver regeneration after PH (23). Gene expression profiles from 12 samples were retrieved from GEO database. The samples were then divided into the SHAM and 70% PH groups (Fig 4A). Volcano plot analyses were used to visualize differentially expressed genes (Fig 4B). Functional enrichment analysis was conducted to analyze differentially expressed genes (DEGs) functions. Gene Ontology (GO) analysis showed that the DEGs were implicated in “DNA replication” and “nuclear division” (Fig 4C). KEGG pathway analysis displayed a significant involvement in cell cycle signaling and DNA replication pathways (Fig 4D-E). Among the DEGs, RRM2

(Ribonucleotide Reductase Regulatory Subunit M2) elicited our attention. RRM2 provides DNA synthesis precursors and accelerates biosynthesis of deoxyribonucleotides from the corresponding ribonucleotides. RRM2 is the primary target for miR-139-5p. Therefore, we hypothesized that circ-RBM23 might regulate RRM2 via the miR-139-5p.

First, we showed that exosome administration increased RRM2, p-AKT, p-mTOR and eIF4G expression, indicating that hPDSCs-exo might regulate cell proliferation via AKT/mTOR pathway (Fig 5A). Next, we found that miR-139-5p mimic downregulated RRM2 expression while miR-139-5p inhibitor upregulated RRM2 expression in L02 cells. A similar expression pattern was noted in p-AKT, p-mTOR and eIF4G (Fig 5B). Finally, it was showed that miR-139-5p inhibited AKT, mTOR and eIF4G expression, and this inhibition was blocked by RRM2 overexpression in L02 cells (Fig 5C). circ-RBM23 upregulated the expression levels of RRM2, p-AKT, p-mTOR and eIF4G, but this upregulation was inhibited by miR-139-5p in L02 cells (Fig 5D). Binding sites between circ-RBM23, miR-139-5p, and RRM2 were summarized in supplementary Table 4.

3.6 Exosomal circ-RBM23 regulated RRM2 via the AKT/mTOR pathway in vivo and in vitro

To determine whether exosomal circ-RBM23 regulated RRM2 in L02 cells, we constructed stable circ-RBM23 siRNA treated hPMSCs, whose exosomes contain low circ-RBM23 expression (Fig 6A). WB analysis showed that co-culturing si-circ-RBM23-exo with L02 cells for 24 h, suppressed the expression of RRM2,

p-AKT, p-mTOR and eIF4G (Fig 6B).

Next, we intraperitoneally injected mice with si-circ-RBM23-exo 24 h prior to 70% PH. WB analysis showed similar results, the expression of RRM2, p-AKT, p-mTOR and eIF4G were downregulated in si-circ-RBM23-exo treated mice (Fig 6C). H&E staining showed that the mitotic count was significantly lower in si-circ-RBM23-exo mice than hPDSCs-exo treated mice detected in the hepatocellular area. In the other hand, PCNA IHC staining showed a similar trend in si-circ-RBM23-exo treated mice compared to the hPDSCs-exo treatment mice. Interestingly, liver mitosis and PCNA IHC staining did not differ in si-circ-RBM23-exo treated mice compared with the controls (Fig 6D).

4. Discussion

This study described a novel and significant function of hPMSCs secreted exosomes. Our findings revealed that hPDSCs-exo directly fused with hepatocytes, promoted their proliferation *in vitro* and *in vivo*. Mechanistically, hPMSCs exosomal circ-RBM23 regulated hepatocyte proliferation by sponging miR-139-5p via RRM2/AKT/mTOR signaling. These results improved our current understanding of hPDSCs-exo and highlight their therapeutic potential against liver disease.

CircRNA is a special type of ncRNA with a stable structure and conserved sequence in different biological tissues and cells (15, 16). Studies indicate that circRNA have critical roles in various diseases, including colorectal cancer, pulmonary fibrosis, and diabetes . We found that circ-RBM23 was a potential key circRNA in hPDSCs-exo, and regulated hepatocyte cell proliferation. In 2011,

Salmena(24-28) et al. suggested a regulatory mechanism of competing endogenous RNAs (ceRNA) (19). Our luciferase reporter assay indicated an interaction between miR-139-5p and circ-RBM23 in L02 cells. RIP assay showed that circ-RBM23 interacted with miR-139-5p. These findings suggested that circ-RBM23 could sponges miR-139-5p to inhibit its function in mouse after PH.

RRM2 comprises two 44-kDa proteins only expressed during late G1/early S phase of cell cycle (29). RRM2 overexpression is linked to cancer cell invasiveness, metastasis, tumorigenesis, and poor patient outcome (29, 30). Our analysis revealed that RRM2 regulates PH development and promotes liver proliferation.

Several studies have recently showed that MSC exosomes have hepatoprotective effect (31-33). Du et al. reported that hiPSC-MSCs-Exo administration immediately after reperfusion harbored hepatoprotective effect (34). MSCs-exo increased hepatocyte proliferation after liver injury via carbon tetrachloride (35, 36). Similarly, we observed a proliferative effect of hPDSCs-exo in PH mice. More importantly, our results demonstrated that hPMSCs exosomal circ-RBM23 induced hepatocyte proliferation and activated AKT/mTOR/eIF4G pathway by sponging miR-139-5p and modulated RRM2 in PH mice. In contrast, downregulated exosomal circ-RBM23 had no effect in hepatocyte proliferation relative to control mice. These findings suggested that hPDSCs-exo promoted their proliferation, which was in line with previous studies.

In summary, for the first time, we describe the proliferative and protective impacts of hPDSCs-exo on hepatocytes post PH. Furthermore, we find that exosomal

circ-RBM23 promotes hepatocyte proliferation via miR-139-5p/RRM2/AKT/mTOR signaling (Fig 7). These results suggest a new mechanism of liver regeneration, which provide a potential therapeutic approach against liver disease and in transplantation.

5. Conclusion

Liver regeneration after partial hepatectomy is complex and well-orchestrated, hPDSCs-exo are membrane nano vesicles (30-100 nm) secreted by hPMSCs. This study shown that the hPMSCs exosomal circ-RBM23 serve as efficient miR-139-5p sponges and regulate liver regeneration of mouse after 70% partial hepatectomy by regulated RRM2/mTOR pathway. This highlighted the importance of ceRNA mechanism in pathogenesis and therapeutic development for liver regeneration in the future.

Declarations

Ethics approval and consent to participate

The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. Experiments were performed under a project license Certificate of Conformity: SYXK (YUE) 2019-0215) granted by The institutional animal care and use committee center of Zhu Jiang Hospital, Southern Medical University, Guangzhou, China, in compliance with China national guidelines for the care and use of animals.

Consent for publication

Not applicable.

Availability of data and materials

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Author contributions

All authors contributed to the study conception and design. The research was designed by YG and TL. Material preparation and data collection were performed by GH, YF, ZG, HZ, HL, XN, LF, YZ and MP. Data analysis was performed by SF, TL, YL, SL and LW. The first draft of the manuscript was written by GH and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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References

1. Michalopoulos GK. Principles of liver regeneration and growth homeostasis. *Compr Physiol*. 2013;3(1):485-513.
2. Fausto N, Campbell JS, Riehle KJ. Liver regeneration. *Hepatology*. 2006;43(2 Suppl 1):S45-53.
3. Guerin DJ, Kha CX, Tseng KA. From Cell Death to Regeneration: Rebuilding After Injury. *Front Cell Dev Biol*. 2021;9:655048.
4. Michalopoulos GK, Bhushan B. Liver regeneration: biological and pathological mechanisms and implications. *Nature reviews Gastroenterology & hepatology*. 2021;18(1):40-55.
5. Duncan AW, Dorrell C, Grompe M. Stem cells and liver regeneration. *Gastroenterology*. 2009;137(2):466-81.
6. Abumaree MH, Abomaray FM, Alshehri NA, Almutairi A, AlAskar AS, Kalionis B, et al. Phenotypic and Functional Characterization of Mesenchymal Stem/Multipotent Stromal Cells From Decidua Parietalis of Human Term Placenta. *Reprod Sci*. 2016;23(9):1193-207.
7. Trounson A, McDonald C. Stem Cell Therapies in Clinical Trials: Progress and Challenges. *Cell stem cell*. 2015;17(1):11-22.
8. Barzegar M, Wang Y, Eshaq RS, Yun JW, Boyer CJ, Cananzi SG, et al. Human placental mesenchymal stem cells improve stroke outcomes via extracellular

vesicles-mediated preservation of cerebral blood flow. *EBioMedicine*. 2021;63:103161.

9. Bai L, Sun L, Chen W, Liu KY, Zhang CF, Wang F, et al. Evidence for the existence of CD34(+) angiogenic stem cells in human first-trimester decidua and their therapeutic for ischaemic heart disease. *J Cell Mol Med*. 2020;24(20):11837-48.
10. Chen J, Shehadah A, Pal A, Zacharek A, Cui X, Cui Y, et al. Neuroprotective effect of human placenta-derived cell treatment of stroke in rats. *Cell Transplant*. 2013;22(5):871-9.
11. Liu H, Honmou O, Harada K, Nakamura K, Houkin K, Hamada H, et al. Neuroprotection by PlGF gene-modified human mesenchymal stem cells after cerebral ischaemia. *Brain*. 2006;129(Pt 10):2734-45.
12. Pegtel DM, Gould SJ. Exosomes. *Annu Rev Biochem*. 2019;88:487-514.
13. Kalluri R, LeBleu VS. The biology, function, and biomedical applications of exosomes. *Science*. 2020;367(6478).
14. Mathieu M, Martin-Jaular L, Lavieu G, Thery C. Specificities of secretion and uptake of exosomes and other extracellular vesicles for cell-to-cell communication. *Nature cell biology*. 2019;21(1):9-17.
15. Han B, Chao J, Yao H. Circular RNA and its mechanisms in disease: From the bench to the clinic. *Pharmacol Ther*. 2018;187:31-44.
16. Kristensen LS, Andersen MS, Stagsted LVW, Ebbesen KK, Hansen TB, Kjems J. The biogenesis, biology and characterization of circular RNAs. *Nat Rev Genet*. 2019;20(11):675-91.
17. Guo X, Xi L, Li L, Guo J, Jin W, Chang C, et al. circRNA-14723 promotes hepatocytes proliferation in rat liver regeneration by sponging rno-miR-16-5p. *J Cell Physiol*.

2020;235(11):8176-86.

18. Lutgehetmann M, Volz T, Kopke A, Broja T, Tigges E, Lohse AW, et al. In vivo proliferation of hepadnavirus-infected hepatocytes induces loss of covalently closed circular DNA in mice. *Hepatology*. 2010;52(1):16-24.
19. Salmena L, Poliseno L, Tay Y, Kats L, Pandolfi PP. A ceRNA hypothesis: the Rosetta Stone of a hidden RNA language? *Cell*. 2011;146(3):353-8.
20. Yi X, Chen F, Liu F, Peng Q, Li Y, Li S, et al. Comparative separation methods and biological characteristics of human placental and umbilical cord mesenchymal stem cells in serum-free culture conditions. *Stem cell research & therapy*. 2020;11(1):183.
21. Mitchell C, Willenbring H. A reproducible and well-tolerated method for 2/3 partial hepatectomy in mice. *Nat Protoc*. 2008;3(7):1167-70.
22. Li T, Weng J, Zhang Y, Liang K, Fu G, Li Y, et al. mTOR direct crosstalk with STAT5 promotes de novo lipid synthesis and induces hepatocellular carcinoma. *Cell Death Dis*. 2019;10(8):619.
23. He G, Fu S, Li Y, Li T, Mei P, Feng L, et al. TCGA and ESTIMATE data mining to identify potential prognostic biomarkers in HCC patients. *Aging (Albany NY)*. 2020;12.
24. Xu H, Wang C, Song H, Xu Y, Ji G. RNA-Seq profiling of circular RNAs in human colorectal Cancer liver metastasis and the potential biomarkers. *Molecular cancer*. 2019;18(1):8.
25. Chen LY, Wang L, Ren YX, Pang Z, Liu Y, Sun XD, et al. The circular RNA circ-ERBIN promotes growth and metastasis of colorectal cancer by miR-125a-5p and miR-138-5p/4EBP-1 mediated cap-independent HIF-1alpha translation. *Molecular cancer*.

2020;19(1):164.

26. Qi F, Li Y, Yang X, Wu Y, Lin L, Liu X. Hsa_circ_0044226 knockdown attenuates progression of pulmonary fibrosis by inhibiting CDC27. *Aging (Albany NY)*.

2020;12(14):14808-18.

27. Shan K, Liu C, Liu BH, Chen X, Dong R, Liu X, et al. Circular Noncoding RNA HIPK3 Mediates Retinal Vascular Dysfunction in Diabetes Mellitus. *Circulation*.

2017;136(17):1629-42.

28. Stoll L, Rodriguez-Trejo A, Guay C, Brozzi F, Bayazit MB, Gattesco S, et al. A circular RNA generated from an intron of the insulin gene controls insulin secretion. *Nature communications*. 2020;11(1):5611.

29. Lopez-Contreras AJ, Specks J, Barlow JH, Ambrogio C, Desler C, Vikingsson S, et al. Increased Rrm2 gene dosage reduces fragile site breakage and prolongs survival of ATR mutant mice. *Genes Dev*. 2015;29(7):690-5.

30. Gandhi M, Gross M, Holler JM, Coggins SA, Patil N, Leupold JH, et al. The lncRNA lincNMR regulates nucleotide metabolism via a YBX1 - RRM2 axis in cancer. *Nature communications*. 2020;11(1):3214.

31. Li X, Liu R, Huang Z, Gurley EC, Wang X, Wang J, et al. Cholangiocyte-derived exosomal long noncoding RNA H19 promotes cholestatic liver injury in mouse and humans. *Hepatology*. 2018;68(2):599-615.

32. Wei X, Zheng W, Tian P, Liu H, He Y, Peng M, et al. Administration of glycyrrhetic acid reinforces therapeutic effects of mesenchymal stem cell-derived exosome against acute liver ischemia-reperfusion injury. *J Cell Mol Med*. 2020;24(19):11211-20.

-
33. Shao M, Xu Q, Wu Z, Chen Y, Shu Y, Cao X, et al. Exosomes derived from human umbilical cord mesenchymal stem cells ameliorate IL-6-induced acute liver injury through miR-455-3p. *Stem cell research & therapy*. 2020;11(1):37.
 34. Du Y, Li D, Han C, Wu H, Xu L, Zhang M, et al. Exosomes from Human-Induced Pluripotent Stem Cell-Derived Mesenchymal Stromal Cells (hiPSC-MSCs) Protect Liver against Hepatic Ischemia/ Reperfusion Injury via Activating Sphingosine Kinase and Sphingosine-1-Phosphate Signaling Pathway. *Cell Physiol Biochem*. 2017;43(2):611-25.
 35. Jiang W, Tan Y, Cai M, Zhao T, Mao F, Zhang X, et al. Human Umbilical Cord MSC-Derived Exosomes Suppress the Development of CCl4-Induced Liver Injury through Antioxidant Effect. *Stem cells international*. 2018;2018:6079642.
 36. Tan CY, Lai RC, Wong W, Dan YY, Lim SK, Ho HK. Mesenchymal stem cell-derived exosomes promote hepatic regeneration in drug-induced liver injury models. *Stem cell research & therapy*. 2014;5(3):76.

Abbreviations: hPDSCs-exo, human placental-derived stem cells exosomes; PH, partial hepatectomy; hPMSCs, human placental mesenchymal stem cells; circ-RNAs, circular RNAs; RRM2, ribonucleotide reductase regulatory subunit M2; AKT, protein kinase B; mTOR, mammalian target of rapamycin; eIF4G, eukaryotic translation initiation factor 4G.

Figure legends

Figure 1. Identification of hPMSCs and hPDSCs-exo. a) Flow cytometry analysis of surface markers of hPMSCs. b) Transmission electron microscopy image of hPDSCs-exo (scale bar, 100nm). c) NTA of hPDSCs-exo (mean: 130nm). d) Western blot for CD9, CD63, CD81 and HSP70 in hPDSCs-exo. Western blots were quantified.

Figure 2. hPDSCs-exo promote liver regeneration *in vivo* and hepatocyte proliferation *in vitro*. a) Liver tissues were collected and followed by H&E staining of the liver postoperative 24h and 48h. b) The number of mitotic hepatocytes was

counted. c) PCNA staining 24h and 48h after surgery. d) The PCNA-positive cells were counted. e) Serum ALT and AST levels were determined. f) Immunofluorescent images of PKH26 (red) together with DAPI for nuclei (blue). g) Cell viabilities were measured by CCK8 in AML12 and L02 cells treated with hPDSCs-exo at the concentrations of 0%, 10%, 20%, 30% and 40% for 24h. Error bars represent the mean±standard error of the mean (SEM). *P<0.05, ***P<0.001.

Figure 3. hPDSCs-exo circ-RBM23 serve as a sponge for miR-139-5p. a)

High-throughput sequencing showed the top 14 expression level of circRNA in hPDSCs-exo, circ-RBM23 was the highest. b) Related RNA expression level (qPCR) of the top 5 circRNA in hPDSCs-exo. c) qPCR analysis showed that linear RNA can be digested by RNase R and β -actin expression was significantly decreased, while the circ-RBM23 has not been affected significantly. d) Sanger sequencing and circPrimer2.0 were used to confirm the characteristic of circ-RBM23 in hPMSCs. e) Related RNA expression level of the circ-RBM23 in L02 cells treated with

circ-RBM23-siRNA for 72h. f) Cell viabilities were measured by CCK8 in L02 cells treated with circ-RBM23-siRNA for 72h. g) Relative RNA expression level of indicated miRNAs after L02 cells were co-transfected with circ-RBM23-siRNA. h) Relative luciferase activity was detected after L02 cells were co-transfected with circ-RBM23 promoter and circ-RBM23-siRNA. i) RIP experiment was performed using the anti-Ago2 or IgG antibody to immunoprecipitates, the expressions of circ-RBM23 and miR-139-5p were measured by qPCR. Error bars represent the SEM. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Figure 4. The key gene in liver regeneration after PH. a) Hierarchical cluster analysis. This analysis correctly classified 5 PH samples (red lines) and 7 control samples (blue lines). b) Volcano plot for the differential express genes. Red points and green points represent DEGs that are statistically significant ($P < 0.05$) and fold change > 1 . RRM2 was in the red point area represent up-regulated gene. c) GO term analysis for all DEGs. Top 8 GO terms were showed ($P < 0.05$). BP (Biological Process), CC(Cellular

Component), MF(Molecular Function) . d) KEGG pathway analysis for all DEGs. Top 10 pathways were showed in the figure (P <0.05). e) The other bubble plot of signaling pathways analyses for DEGs. The rich factor indicates the ratio of DEG numbers annotated of total gene numbers annotated in this pathway group. Larger rich factor represents bigger degree of pathway enrichment. The $FDR \leq 0.05$ means significant pathway enrichment. DEGs: Differentially Expressed Genes. GO: gene ontology. FDR: False discovery rate.

Figure 5. Circ-RBM23 regulates hepatocytes proliferation via miR-139-5p/Rrm2/mTOR pathway *in vivo* and *in vitro*. a) Liver tissues were lysed from hPDSCs-exo or vehicle treated PH mice, RRM2 and mTOR pathway protein levels were determined by western blots. Western blots were quantified. b-d) RRM2 and mTOR pathway protein levels were determined by western blots in indicate treated L02 cells; miR-139-5p inhibitor, miR-139-5p mimic; miR-139-5p up; circ-RBM23 up. Western blots were quantified. Error bars represent the SEM. * $P < 0.05$.

Figure 6. Exosomal circ-RBM23 regulates liver regeneration via miR-139-5p/RRM2/mTOR pathway. a) Related expression level (qPCR) of the circ-RBM23 in exosomes derived from hPMSCs treated with circ-RBM23-siRNA for 72h. b) Cells were lysed from circ-RBM23-siRNA treated hPDSCs-exo or vehicle treated L02 cells, RRM2 and mTOR pathway were determined by western blots. Western blots were quantified. c) Liver tissues were lysed from circ-RBM23-siRNA pretreated hPDSCs-exo or vehicle treated PH mice, RRM2 and mTOR pathway protein levels were determined by western blots. Western blots were quantified. d) Liver tissues were collected and followed by H&E and PCNA staining of the liver postoperative from hPDSCs-exo, circ-RBM23-siRNA pretreated hPDSCs-exo or vehicle treated PH mice. The number of mitotic hepatocytes and PCNA-positive cells were counted. Error bars represent the SEM. * $P < 0.05$, *** $P < 0.001$.

Figure 7. Schematic diagram of the mechanism of exosomal circ-RBM23 in liver regeneration. The hPMSCs release contained circ-RBM23 exosomes, which were endocytosed and taken up by hepatocytes. The circ-RBM23 exosomes activates the circ-RBM23/miR-139-5p/RRM2/mTOR pathway to promote liver regeneration.

Supplementary Files

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- [supplementarymaterialsandmethods.pdf](#)