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

ting li
Department of Hepatobiliary Surgery, Zhujiang Hospital, Southern Medical University
yu fu
Department of Hepatobiliary Surgery, Zhujiang Hospital, Southern Medical University
zeyi guo
Department of Hepatobiliary Surgery, Zhujiang Hospital, Southern Medical University
honglei zhu
Department of gynaecology and obstetrics, Zhujiang Hospital, Southern Medical University
hangyu liao
Department of Hepatobiliary Surgery, Zhujiang Hospital, Southern Medical University
xiaoge niu
Department of Special medical service center, Zhujiang Hospital, Southern Medical University
lin zhou
State Key Laboratory of Transducer Technology, Shanghai Institute of Microsystem and Information Technology, Chinese Academy of Sciences
shunjun fu
Department of Hepatobiliary Surgery, Zhujiang Hospital, Southern Medical University
shao li
Department of Hepatobiliary Surgery, Zhujiang Hospital, Southern Medical University
lujia wang
The Second School of Clinical Medicine, Southern Medical University
yizhou zheng
School of Public Health, China Medical University
mingxin pan
Department of Hepatobiliary Surgery, Zhujiang Hospital, Southern Medical University
yi gao
Department of Hepatobiliary Surgery, Zhujiang Hospital, Southern Medical University
guolin he (dwtou@126.com)
Department of Hepatobiliary Surgery, Zhujiang Hospital, Southern Medical University

Research Article

Keywords: Exosomes, circ-RBM23, placental stem cells, liver regeneration, ceRNA mechanism

Posted Date: March 8th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-783482/v2

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-derived mesenchymal stem cells (hPMSCs) were isolated and characterized. Cell co-culture model was used to clarify whether exosomes can induce hepatocyte proliferation *in vitro*. Partial hepatectomy mouse model was used to evaluate whether exosomes can promote hepatocytes proliferation *in vivo*.

Results

Our results demonstrated that human placental-derived stem cells exosomes (hPMSCs-exo) can induce hepatocyte proliferation *in vitro* and *in vivo*. Mechanistically, exosomal circ-RBM23 served as a ceRNA (competing endogenous RNAs) for miR-139-5p, regulated RRM2 and accelerated proliferation through AKT/mTOR pathways. Ablation of circ-RBM23 suppressed the pro-proliferative effect of exosomes.

Conclusions

The hPMSCs exosomal circ-RBM23 stimulated cell proliferation and liver regeneration after 70% partial hepatectomy by a ceRNA network mechanism. Our findings highlight a potential novel therapeutic avenue for liver regeneration.

1. Introduction

Liver regeneration is a compensatory process replacing 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 a complex and well-orchestrated process, involving multiple factors including cytokines, exosomes, growth factors, and signaling pathways (2–5). Identifying the mechanism and regulation of liver regeneration significantly helps liver resection patients.

Human placental-derived mesenchymal stem cells (hPMSCs) exhibit self-renewing potential and are capable of differentiating into multiple cell type lineages (6). They secrete soluble factors with paracrine effects. The therapeutic potential of hPMSCs has been demonstrated in tissue damage repair after

ischemic disorders including stroke, and myocardial and cerebral infarctions (7–11). These studies ascertain 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 which may potentially influence 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 revealed that circ-RNAs act as efficient miRNA sponges regulating liver regeneration (17–19). Nonetheless, the roles of mesenchymal stem cells especially hPMSCs exosomal circ-RNAs and their function in liver regeneration is not completely understood.

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

2. Results

2.1 Identification of hPMSCs and hPMSCs-exo

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

2.2 hPMSCs-exo promoted liver regeneration in vivo and hepatocyte proliferation in vitro

To determine whether hPMSCs-exo triggers hepatocyte regeneration *in vivo*, we administered (1×10^8 Particles/mL) hPMSCs-exo 24 h before 70% PH surgery by the tail veins injection. Relative to PH-only mice, exosomes treatment significantly increased liver mitosis 2 days after PH as indicated by H&E staining (Fig. 2A-B). Additionally, PCNA IHC staining showed that exosomes promoted cell proliferation in mice subjected to PH (Fig. 2C-D). Furthermore, we explored that compare to exosomes treated mice, untreated mice exhibited obviously higher levels of ALT and AST (Fig. 2E). To determine whether hPMSCs-exo induce hepatocyte proliferation, we co-cultured AML-12 and L02 cells with hPMSCs-exo which labeled with PKH26 (a cell membrane marker for label exosome). Confocal analysis showed that exosomes were directly absorbed by cells (Fig. 2F). Moreover, CCK8 assays showed elevated cell

proliferation after hPMSCs-exo treatment for 24 h (Fig. 2G). These results indicating that hPMSCs-exo harbor a pro-proliferative and protective role in PH mice.

2.3 The identification and characteristics of circ-RBM23 in hPMSCs-exo

Circular RNA expression in hPMSCs-exo was profiled using high-throughput sequencing. 14 possible circular RNAs were selected based on their higher expression (Fig. 3A). Among these circular RNAs, circ-RBM23, circ-SWAP70, circ-MAPK9, circ-FKBP8, and circ-EPHB4 were highly expressed in hPMSCs-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 selected for subsequent analysis. Primer of linear RBM23 and circ-RBM23 were showed in supplementary Table 2. Compare to linear RBM23, circ-RBM23 was more resistant to RNase R digestion (Fig. 3C). Furthermore, Sanger sequencing and circPrimer2.0 were used to verify the head-to-tail splicing, circular features and characterized circ-RBM23 expression in hPMSCs (Fig. 3D). siRNA assay was used to knock down circ-RBM23 in L02 cells, this was confirmed by RT-qPCR assay ($p < 0.01$, Fig. 3E). CCK8 assay also showed that downregulating circ-RBM23 inhibited the proliferation of L02 cells (Fig. 3F).

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

Previous studies indicated that circular RNA primarily functions as miRNA sponges. Then a rough miRNA target prediction was made for circ-RBM23 using a bioinformatics tool starBase2.0 (https://starbase.sysu.edu.cn/). The top-5 potential circ-RBM23 target miRNAs and their primer were list in supplementary Table 3. RT-qPCR analysis showed that circ-RBM23 silencing upregulated miRNA expression, and miR-139-5p was the most significantly upregulated (Fig. 3G). Dual-luciferase reporter analysis identified 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 more enricher in Ago2-containing immunoprecipitates than actin or miR-139-5p (Fig. 3I), this indicates that circ-RBM23 molecularly sponges miR-139-5p. Identically, compared to sham-operated mice, we found that the expression of circ-RBM23 was increased in mice undergoing PH while the expression of miR-139-5p was decreased (Fig S1A,B).

2.5 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 extracted from the GEO database. The samples were then divided into the control group and LR (liver resection, 70% PH) group (Fig. 4A). Volcano plot analysis was 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 of

DEGs 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 is one of target for miR-139-5p, it provides DNA synthesis precursors and accelerates biosynthesis of deoxyribonucleotides from the corresponding ribonucleotides. Huang et al. reported that RRM2 was binding with miR-139-5p by a dual luciferase reporter assay in Non-small cell lung cancer cells (24). Our data also showed that RRM2 was the target gene of miR-139-5p and binding together in L02 cells (Fig S1C). Therefore, we hypothesized that circ-RBM23 might regulate RRM2 via the miR-139-5p.

Administration of hPMSCs-exo upregulated the expression of RRM2, p-AKT, p-mTOR and eIF4G in L02 cells, indicating that hPMSCs-exo might regulate cell proliferation via AKT/mTOR pathway (Fig. 5A). Further, we found that miR-139-5p mimic downregulated RRM2 expression while miR-139-5p inhibitor upregulated RRM2 expression. A similar expression pattern was observed in p-AKT, p-mTOR and eIF4G (Fig. 5B). Finally, it was showed that miR-139-5p inhibited the expression of AKT, mTOR and eIF4G, 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, however, 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.

2.6 Exosomal circ-RBM23 regulated RRM2 via the AKT/mTOR pathway

Stable si-circ-RBM23 cells, whose exosomes contain low circ-RBM23 expression were constructed to establish whether exosomal circ-RBM23 regulates RRM2 in AML-12 and L02 cells (Fig. 6A). After 24 h co-culture si-circ-RBM23-exo with L02 cells, RT-qPCR analysis showed si-circ-RBM23-exo activated the expression of miR-139-5p (Fig S1D), western blot analysis showed si-circ-RBM23-exo inhibited the expression of RRM2, p-AKT, p-mTOR and eIF4G (Fig. 6B).

The mice were injected with si-circ-RBM23-exo 24 h before 70% PH via tail veins. RT-qPCR analysis showed si-circ-RBM23-exo activated the expression of miR-139-5p (Fig S1E), western blot 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 revealed that the mitotic counts were significantly lower in si-circ-RBM23-exo mice than that in hPMSCs-exo treated mice 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 hPMSCs-exo treated mice. Interestingly, liver mitosis and PCNA IHC staining did not differ in si-circ-RBM23-exo treated mice compared with the controls (Fig. 6D).

3. Discussion

This study described a novel and significant function of hPMSCs secreted exosomes. The results show that hPMSCs-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. This improves our current understanding of hPMSCs-exo and highlights 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 indicated that circRNA has critical roles in various liver diseases, including metabolic-associated fatty liver disease, hepatitis, liver cirrhosis and hepatocellular carcinoma (25). Li et al. demonstrated the existence of abundant exo-circRNAs and serum exo-circRNA may as a circulating biomarker for cancer diagnosis (26). MSC-derived exosomes carry complex cargo, including nucleic acids, proteins, and lipids. Previous research indicated that exosome cargo are play important role in modulate recipient cell behaviors of liver diseases. In the report of Shao et al, they indicated that human umbilical cord mesenchymal stem cells derived miR-455-3p-enriched exosomes are able to suppress monocyte/macrophage activation and alleviate acute liver injury by inhibiting IL-6-related signaling pathways (27). Zhu et al. showed that exosomes derived from circ_0000623-modified adipose-derived mesenchymal stem cells prevent liver fibrosis (28). But none of the studies has reported about exo-circRNAs in liver regeneration. Here, we discovered that circ-RBM23 is a potentially key circRNA in hPMSCs-exo, and regulated hepatocyte cell proliferation. In 2011, Salmena et al. reported a regulatory mechanism of competing for 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 sponge miR-139-5p to inhibit its function in hepatocytes during the PH of a mouse.

RRM2 comprises two 44-kDa proteins only expressed during the late G1/early S phase of the cell cycle (29). RRM2 overexpression is linked to cancer cell invasiveness, metastasis, tumorigenesis, and poor patient outcome (30). Our analysis revealed that RRM2 which is targeted by miR-139-5p, regulates the development of PH and promotes liver proliferation.

Several recent studies have demonstrated that MSC exosomes exhibit hepatoprotective effect (27, 31, 32). Du et al. reported that hiPSC-MSCs-Exo administration immediately showed hepatoprotective effects after reperfusion (33). MSCs-exo increased hepatocyte proliferation after liver injury via carbon tetrachloride (34, 35). Similarly, we observed a pro-proliferative effect of hPMSCs-exo in PH mice. More importantly, our results demonstrate that hPMSCs exosomal circ-RBM23 induces hepatocyte proliferation and activates AKT/mTOR/eIF4G pathway by sponging miR-139-5p and modulating RRM2 in PH mice. In contrast, downregulated exosomal circ-RBM23 did not affect in hepatocyte proliferation relative to control mice. These findings suggested that hPMSCs-exo promotes hepatocyte proliferation, which was in line with previous studies.

In summary, for the first time, we described the pro-proliferative and protective impacts of hPMSCs-exo on hepatocytes after PH. Furthermore, we indicate that exosomal circ-RBM23 promoted hepatocyte proliferation via miR-139-5p/RRM2/AKT/mTOR signaling (Fig. 7). Our findings suggest a novel mechanism of liver regeneration, which provide a potential therapeutic approach against liver disease and in transplantation.

4. Conclusion

Liver regeneration after partial hepatectomy is complex and well-orchestrated, hPMSCs-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 via the RRM2/AKT/mTOR pathway. This highlighted the importance of ceRNA mechanism in pathogenesis and therapeutic development for liver regeneration in the future.

5. Materials And Methods

5.1. Human placenta mesenchymal 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.

5.2. RNase R treatment

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

5.3.Exosomes isolation

Notably, 5×10^7 cells were seeded in 25 mL media supplemented with 10% exo-depleted FBS in a 150 cm^2 tissue culture plate for 48 h to extract exosomes from the conditioned medium of cell cultures. 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 excess cells and cellular debris. Then, the supernatant was centrifuged for 10,000 g for 30 min and filtered using a 0.22 μm filter (Merck Millipore, Germany) to eliminate cell debris membranes and EVs. After 2 h of ultracentrifugation at 100,000 g, a pellet was collected 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 then resuspended in PBS for downstream analyses.

5.4.Exosomes characterization

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

5.5.High-throughput sequencing

High-throughput sequencing and OE Biotech (Shanghai, China) were used to determine the circ-RNAs expression profiles. Total RNA was extracted with TRIzol and the RNA purity and concentration were determined, then a cDNA library constructed after removing ribosomal RNA and RNase R digestion. Illumina sequencer (HiSeqTM 2500) was used after passing quality inspection. Eventually, circ-RNAs were confirmed and statistically analyzed.

5.6.Bioinformatics analysis

Data Source: Gene expression profiles were obtained from GEO database (https://www.ncbi.nlm.nih.gov/geo/). Identification of differentially expressed genes (DEGs), Heatmap and clustering analysis: Data were analyzed using the R software (version 3.6.2) and its package limma. |logFC|>1 and p = < 0.05 was set as a 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.

5.7.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 experimental protocols were approved by the institutional animal care and use committee center of Zhu Jiang Hospital (Southern Medical University, Guangzhou, China). The mice were housed in plastic cages at a controlled temperature of 22 ± 1°C. Standard rodent chow and water were provided. All animals and samples were assigned a number that did not expose their treatment group for blind analysis.

5.8.Murine 70% partial hepatectomy model

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

5.9.RNA-binding protein immunoprecipitation (RIP) assay

Immunoprecipitation of the circ-RBM23 bound to Ago2 was performed using a Magna RIP™ RNA-binding protein immunoprecipitation kit (Merck Millipore, Germany). In total, 2×10^7 L02 cells were harvested in RIP lysis buffer and lysates stored at -80℃. Further, 8 μg of anti-Ago2 (MA5-23515, Invitrogen, USA) or

normal control IgG was incubated with magnetic beads at room temperature for 2h 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℃. Thereafter, the beads were mixed with proteinase K buffer and incubated at 55℃ for 30 min, the RNA was extracted for PCR.

5.10.Dual-luciferase report assay

A dual-luciferase reporter assay (Promega, Madison, WI, USA) kit was used to conduct a dual-luciferase assay as usual (22). L02 cells were seeded on 24-well plates and cultured to 50% confluence. They were then transfected with circ-RBM23-wt or circ-RBM23-mut plasmid and negative control renilla luciferase plasmid together with miR-139-5p for 48 h. The same procedure was implemented with miR-139-5p and RRM2. 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, Madison, WI, USA) kit following the manufacturer's instructions.

5.11.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.

5.12.Statistical analysis

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

Abbreviations

hPMSCs-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.

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.

Funding

This work was supported by China Postdoctoral Science Foundation (Grant Number F121ZJ0216); the National Key R&D Program of China (2018YFC1106400); Science and Technology Planning Project of Guangdong Province(2015B020229002); The Natural Science Foundation of Guangdong Province (2014A030312013, 2018A030313128); Guangdong key research and development plan (2019B020234003); The National Natural Science Foundation of China (61971410, 61801464); The College Students' innovation and entrepreneurship training program of the Southern Medical University (201912121205).

Author contributions

All authors contributed to the study conception and design. The research was designed by GH, YG and TL. Material preparation and data collection were performed by TL, YF, ZG, HZ, HL, XN, YZ and MP. Data analysis was performed by SF, TL, SL, LZ 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.

Acknowledgments

We appreciate each participant providing assistance in this study.

Footnote

Addres: Guolin He, Ph.D., Department of Hepatobiliary Surgery II, Zhujiang Hospital, Southern Medical University, 253 Gongye Street, Guangzhou, Guangdong Province, China. E-mail: dwtou@126.com.

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Figures

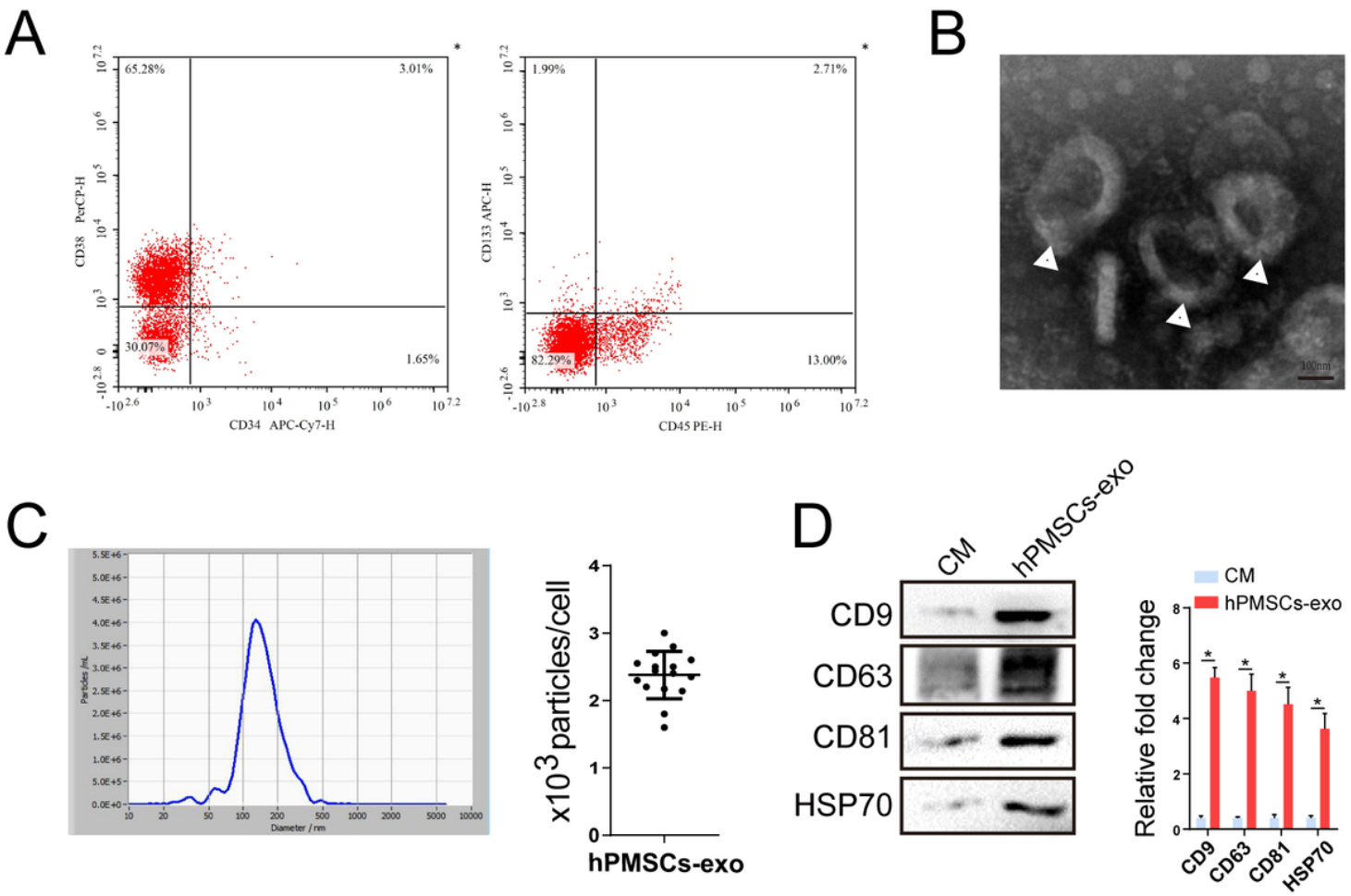

Figure 1

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

Figure 2

hPMSCs-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 hPMSCs-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

hPMSCs-exo circ-RBM23 serve as a sponge for miR-139-5p. a) High-throughput sequencing showed the top 14 expression level of circRNA in hPMSCs-exo, circ-RBM23 was the highest. b) Related RNA expression level (RT-qPCR) of the top 5 circRNA in hPMSCs-exo. c) RT-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 lgG antibody to immunoprecipitates, the expressions of circ-RBM23 and miR-139-5p were measured by RT-qPCR. Error bars represent the SEM. *P<0.05, **P<0.01, ***P<0.001.

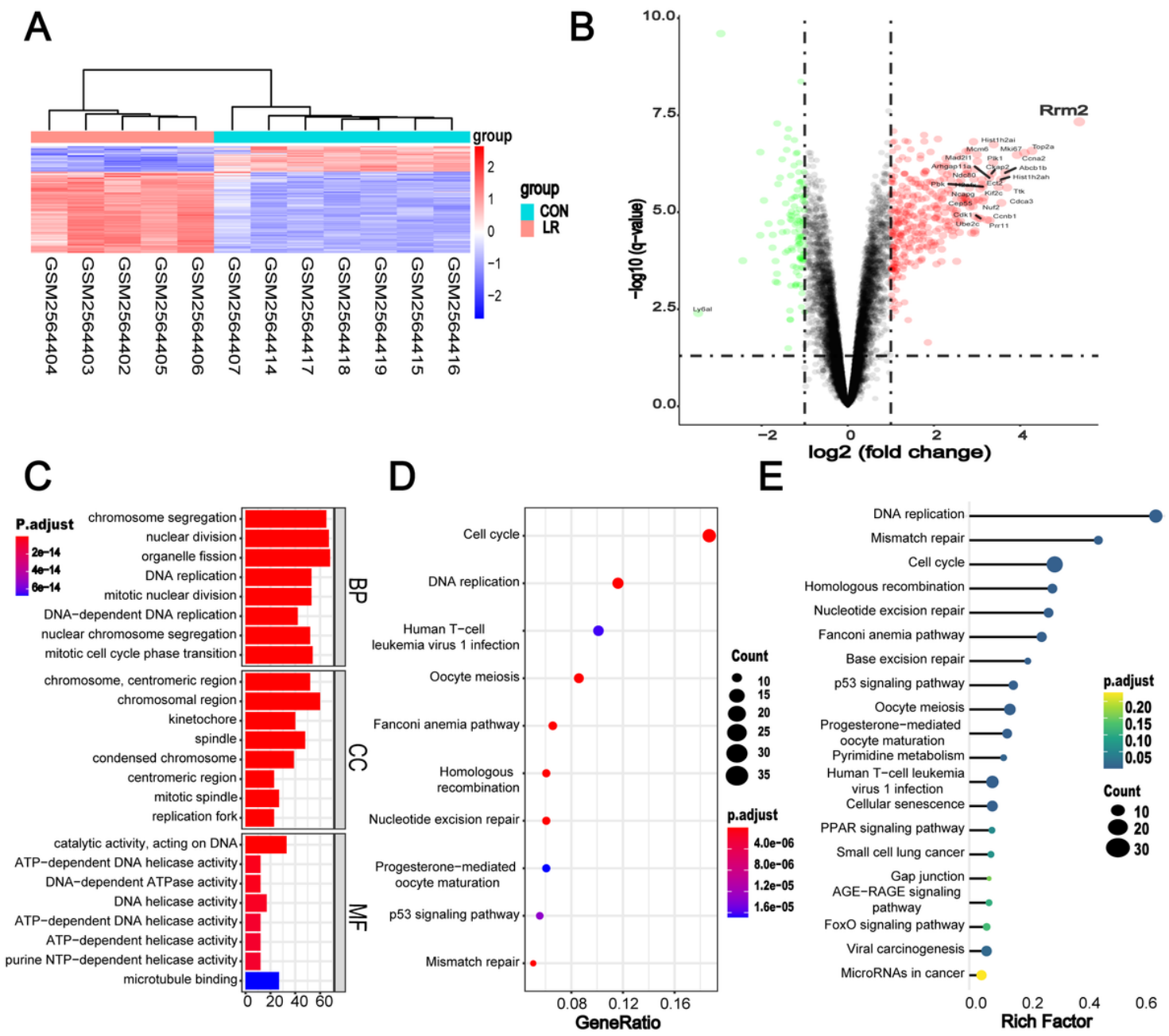

Figure 4

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≤0.05 means significant pathway enrichment. DEGs: Differentially Expressed Genes. GO: gene ontology. FDR: False discovery rate.

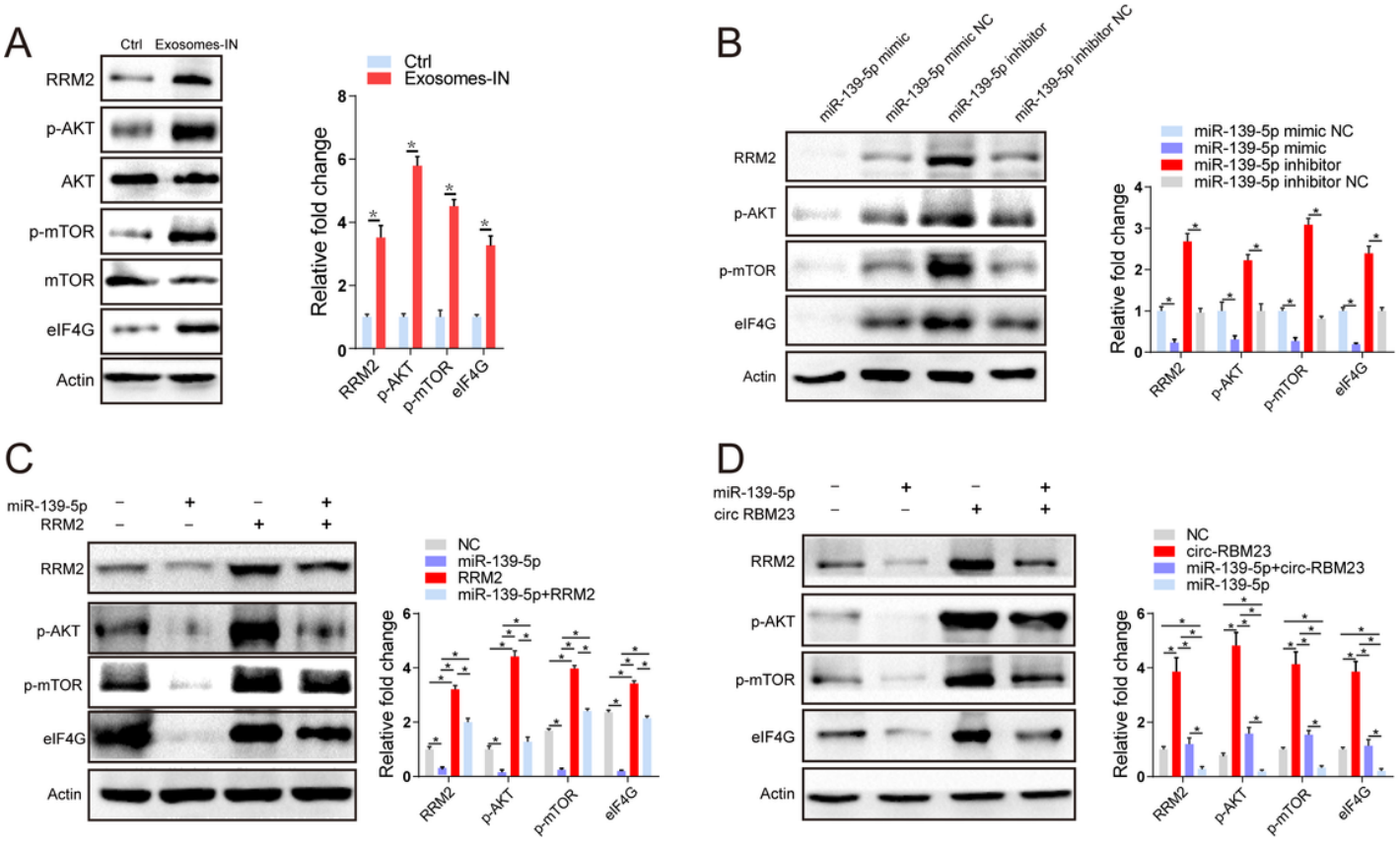

Figure 5

circ-RBM23 regulates hepatocytes proliferation via miR-139-5p/RRM2/AKT/mTOR pathway *in vivo* and *in vitro*. a) Liver tissues were lysed from hPMSCs-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/AKT/mTOR pathway. a) Related expression level (RT-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 hPMSCs-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 hPMSCs-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

hPMSCs-exo, circ-RBM23-siRNA pretreated hPMSCs-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.m

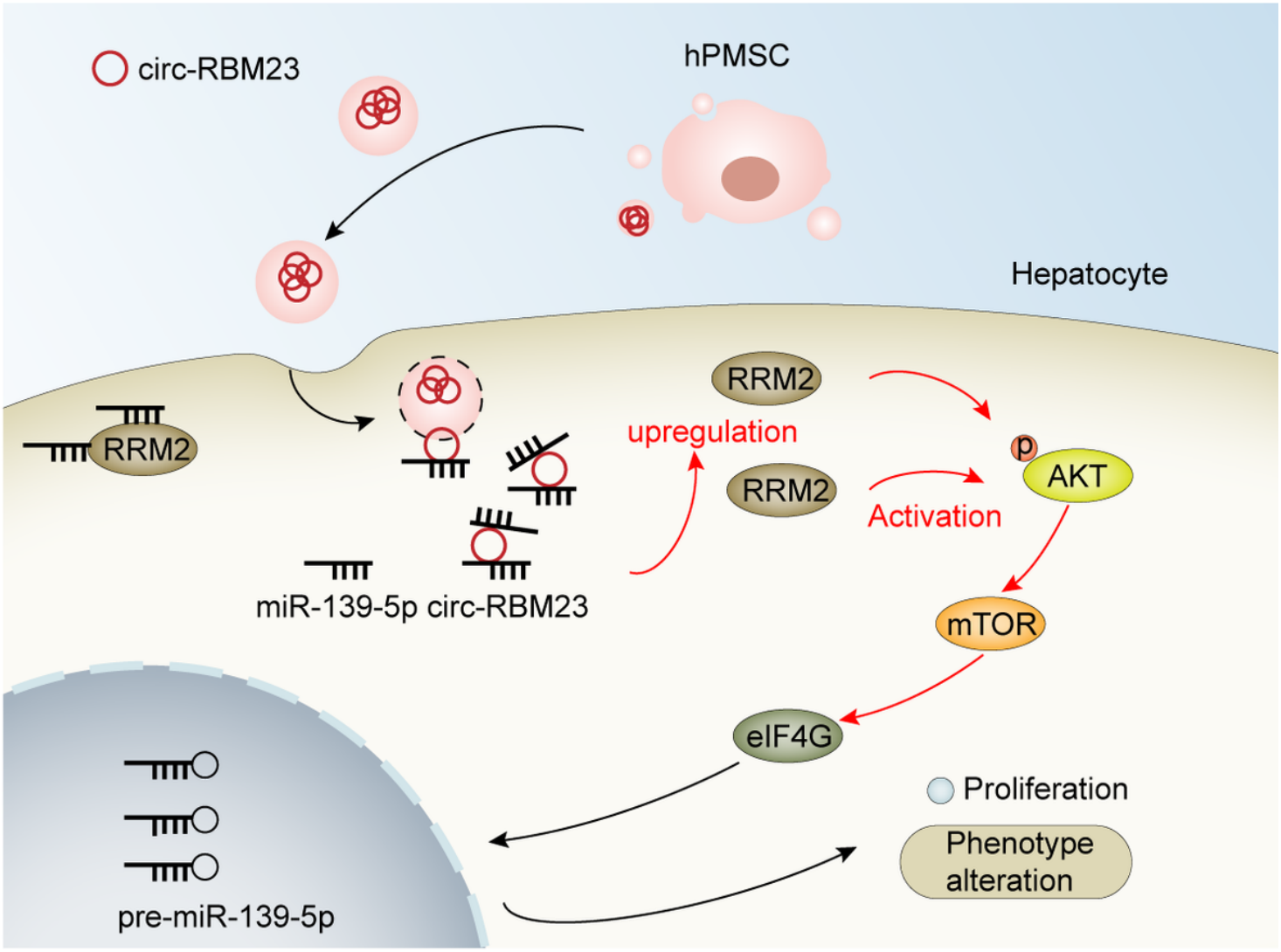

Figure 7

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 miR-139-5p/RRM2/AKT/mTOR pathway to promote liver regeneration.

Supplementary Files

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- supplementarymaterialsandmethods.docx