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Supplementary Data

Supplementary materials and methods

Supplementary Figure 1. Identification of proteinase K digested exosomes.

Supplementary Figure 2. Gel electrophoresis identifying exosomal RNAs.

Supplementary Data 1. Oligonucleotide Sequences used in this study.

Supplementary Data 2. HCV Target Site Prediction of Top 15 Highly Expressed MicroRNAs in uMSC-Exosome.

Supplementary materials and methods

Characterization of uMSC by flow cytometry

The isolated uMSCs were detached from the culture flask, washed and suspended in 1% bovine serum albumin (BSA) containing relevant antibodies including CD29 (Clone MAR4), CD90 (Clone 5E10), CD44 (Clone C26), CD105 (Clone 266), CD73 (Clone AD2), CD34 (Clone 563), CD14 (Clone M5E2), CD45 (Clone HI30), CD19 (Clone HIB19) and HLA-DR (Clone G46-6) (BD Biosciences, CA, USA), and incubated at room temperature for 30 min. The isotype antibody was used as a control. The cells were then washed with the BSA, and analyzed using BD FACScalibur flow cytometry analyzer (BD Biosciences, CA, USA).

Production of cell culture-derived HCV and HCV pseudo-particles

The plasmid encoding Japanese fulminant hepatitis type 1 (JFH-1) was a gift from T. Wakita (National Institute of Infectious Diseases, Tokyo, Japan), and was used to produce cell culture-derived HCV (HCVcc) of JFH-1 as previously described [1]. Briefly, the plasmid of JFH-1 was linearized and used as the template during in vitro transcription assay using MEGAscript kit (Promega, Madison, WI). The Huh7 cells were then electroporated with 10 ng produced viral RNA per 10^6 cells, and the supernatants of the cells were collected 5 days

after electroporation. The virions were further concentrated and purified by 20% sucrose centrifugation at 120,000 x g for 3 h at 4 ^{o}C [2].

HCV pseudo-particles (HCVpp) carrying HCV 1a strain H77 E1/E2 were generated as previously described [3, 4]. Briefly, HEK 293T cells were transfected with the plasmids of HCV envelope proteins, Gag/Pol, Rev and the transfer vector of pLenti6 containing the green fluorescent protein (GFP) gene. The supernatants of the transfected cell were collected 48 h post-transfection and filtered through a 0.45-μm membrane. The plasmids encoding HCV envelope proteins 1a strain H77 was provided by F.L. Cosset (INSERM U758, Lyon, France).

HCV infection assay and HCV entry assay

For HCVcc infection, Huh7 cells were seeded on a 96-well plate the previous day and challenged with HCVcc of JFH-1 for 6 h at 37^{o}C. The supernatants were then removed and fresh media added. The cells were cultured for 48 h before the infection was measured using immunofluorescent (IF) assay as previously described [1].

For HCVpp entry, seeded Huh7 cells were incubated with HCVpp of H77 for 6 h at 37^{o}C before changing the fresh medium. The cells were then cultured for 72 h before the entry rate of HCVpp was measured using flow cytometry according to a previous study [5].

Isolation of RNA and qRT-PCR

Total RNA was extracted from the cells or uMSC-Exo using Trizol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. MiRNA isolation was performed with a Tiangen miRcute Kit (Tiangen, China). The cDNA was synthesized with the PrimeScript™ RT Master Mix (Perfect Real Time) (Takara, China). For miRNA, specific primers for reverse transcription were added during cDNA synthesis. Quantitative real-time polymerase chain reaction (qRT-PCR) was conducted using SYBR® Premix Ex Taq™ (Takara, China). GAPDH was used as mRNA endogenous control.

U6 was used as miRNA endogenous control. The primer sequences of the above genes used were listed in Supplementary Data 1.

Western blotting

Cells were lyzed with RIPA lysis and extraction buffer (Thermo Scientific, Rockford, IL, USA), and total proteins were quantified using BCA protein assay kit (Thermo Scientific Pierce, Rockford, IL, USA). Equal amounts of protein were subjected to 10% SDS-PAGE, and incubated with the antibodies targeting specific proteins. Primary antibodies included anti-CD81 monoclonal antibodies (mAb) (Santa Cruz, Paso Robles, CA, at 1:1000 dilutions), Exosome CD63 Antibody (Ts63) (at 1:1000 dilutions), anti-GAPDH (at 1:2000 dilutions), anti-HCV core mAb (at 1:1000 dilutions) and horseradish peroxidase (HRP) conjugated anti-mouse IgG (at 1:1000 dilutions) (Invitrogen, Carlsbad, CA).

For silver staining of the exosomal protein product, we used Fast Silver Staining kit (Beyotime, Shanghai, China) according to the manufacturer's protocol.

MicroRNA sequencing and analysis

Small RNA sequencing was performed using concentrated uMSC-Exos under the help of Shanghai NovelBio Bio-Pharm Technology Co.,Ltd. Briefly, exosomes total RNAs were extracted and small RNA fragments ranging from 18–30 nt were isolated, purified and subsequently ligated to 3' and 5' adaptors sequentially, reverse transcribed to cDNA and then PCR amplified. The entire library was tested by gel electrophoresis, and bands corresponding to microRNA insertion were cut and eluted. After ethanol precipitation and washing, the purified small RNA libraries were quantified sequenced using the Illumina HiSeq™ 2000 analyzer (Illumina, San Diego, USA) according to the manufacturer's instructions. For analysis of small RNA sequencing data, the clean reads were compared with the Rfam database (ftp://selab.janelia.org/pub/Rfam) to match the known miRNA, rRNA, snoRNA, snRNA and tRNA sequences, and were then compared with the human

mature miRNAs database in miRBase (v21) to identify mature miRNAs and count their reads. The raw counts of miRNA reads were further normalized by transcripts per million (TPM) values ((miRNA total reads/total clean reads) $\times 10^6$). The differentially expressed miRNAs (DEmiRNAs) between samples were identified by the EdgeR program using parameters of $P \leq 0.01$ and fold change ≥ 2 or ≤ 0.5. The sequencing data was deposited to GEO database as GSE69909.

References:

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2. Wakita T, Pietschmann T, Kato T, et al. Production of infectious hepatitis C virus in tissue culture from a cloned viral genome. Nat Med 2005;11:791-796.
3. Guan M, Wang W, Liu X, et al. Three different functional microdomains in the hepatitis C virus hypervariable region 1 (HVR1) mediate entry and immune evasion. J Biol Chem. 2012;287:35631-35645.
4. Tong Y, Zhu Y, Xia X, et al. Tupaia CD81, SR-BI, claudin-1, and occludin support hepatitis C virus infection. J Virol 2011;85:2793-2802.
5. Bartosch B, Dubuisson J, Cosset FL. Infectious hepatitis C virus pseudo-particles containing functional E1-E2 envelope protein complexes. J Exp Med 2003;197:633-642.

Supplementary figures

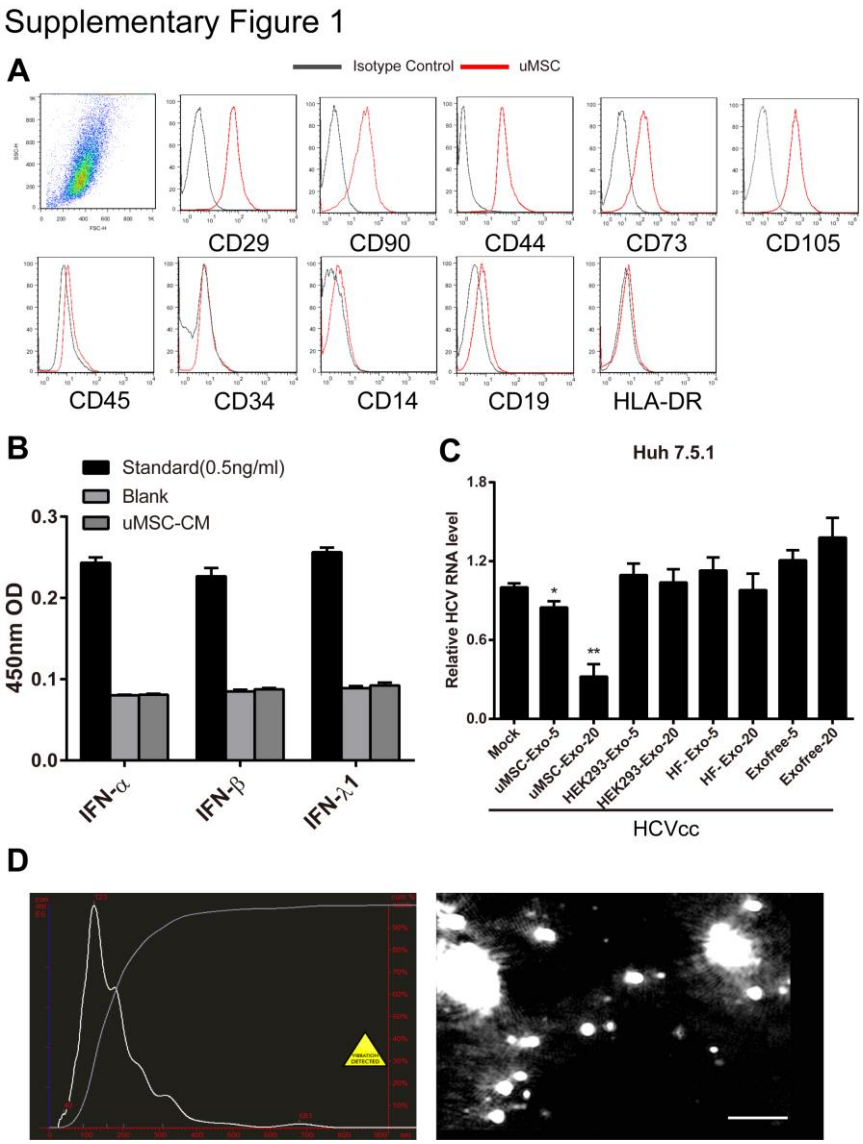

Figure S1.

(**A**): Isolated uMSCs were subjected to flow cytometry to test the expression of CD29, CD90, CD44, CD105, CD73, CD34, CD14, CD45, CD19 and HLA-DR. Isotype antibodies were introduced as a control. (**B**): The supernatant of uMSC (uMSC-CM) was collected to test the levels of IFN-α, IFN-β and IFN-λ1 by ELISA kits. Standard solutions (0.5 ng/ml) and plain culture medium (Blank) were utilized as positive and negative controls. (**C**): Huh7.5.1 cells were infected with HCVcc of JFH-1 (MOI=1) in the presence of different cell types-derived exosomes for 6 h followed by media change with the same amount of exosomes. HCV RNA level was detected 48 h after infection. *, $p<0.05$; **, $p<0.01$ compared with mock control. (**D**): Human uMSC derived exosomes were treated with proteinase K supplemented with Triton X-100 for exosomal protein digestion. Digested exosomes were put to Nanosight analysis to identify its integrity. Representative analysis report of the particle size of digested exosomes were shown. Report showed only particle size peak around 123nm were detected, which indicated that the digested exosomes are still intact. The lower panel showed representative image of detected particles in the sample, which is similar in size with that of undigested exosomes in Figure 2A. The scale bar represented 200 nm.

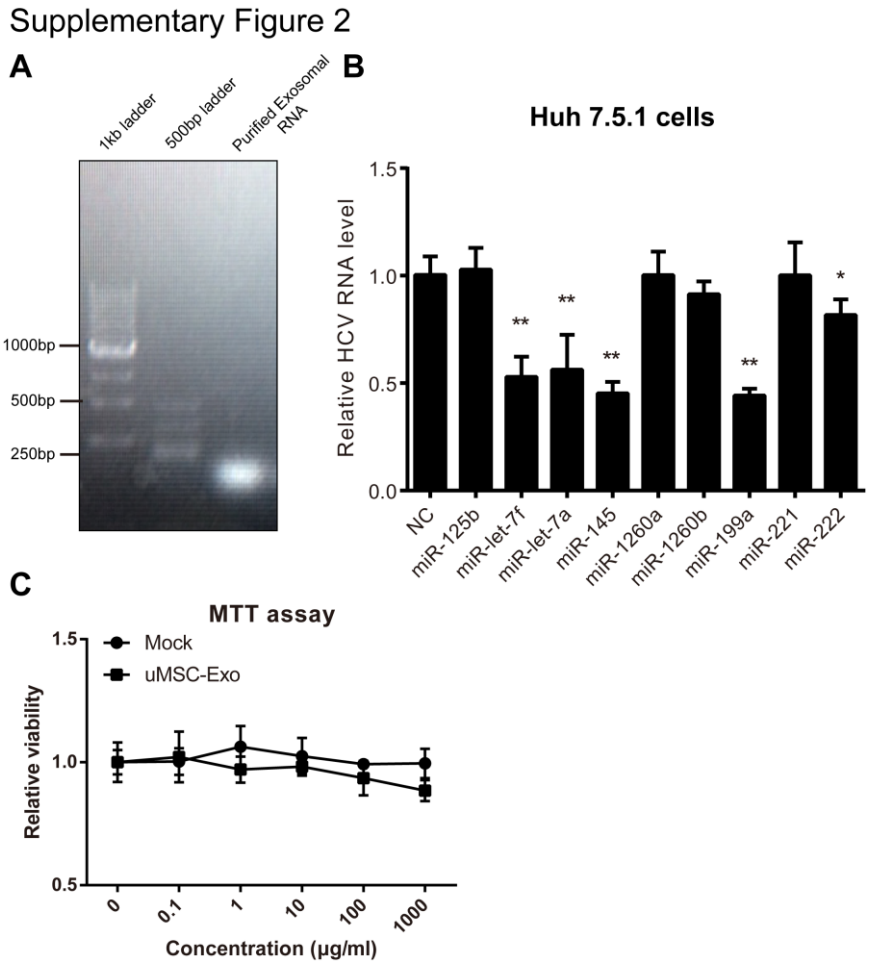

Figure S2.

(**A**): To analysis the RNA components in uMSC derived exosomes, we extracted the total RNA and put to gel electrophoresis analysis using 1% agarose gel with Ethidium Bromide. The result showed that a major band at less than 100bp were detected. (**B**): Huh7.5.1 cells were transfected with chemically synthesized mimics of the 9 miRNAs, and infected with HCVcc of JFH-1. At 48 h after virus inoculation, HCV infection was evaluated in HCV RNA level by RT-qPCR. *, $p<0.05$; **, $p<0.01$ compared with negative control (NC). (**C**): Huh7 cells were incubated with indicated concentrations of uMSC-Exo or solvent (mock) for 24 h before the cell viability was determined by MTT assay.