Supplementary Data

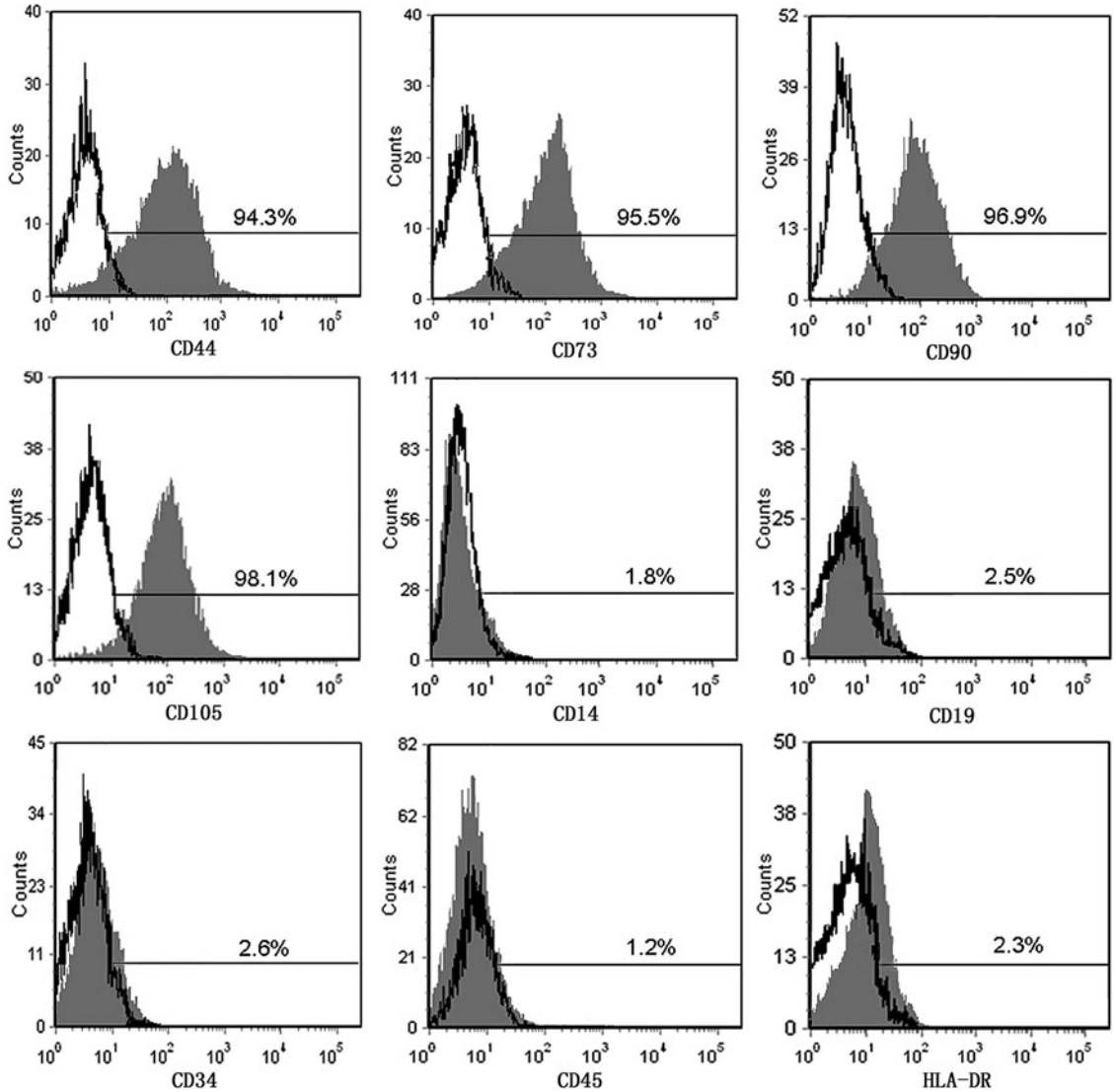
Supplementary Materials and Methods

Flow cytometry analysis

Flow cytometry analysis was performed as previously described.[1] Mesenchymal stem cells from human umbilical cord Wharton's jelly (WJ-MSCs) were incubated at 4°C for 30 min with the following anti-human antibodies, which were conjugated with fluorescein isothiocyanate (FITC) or phycoerythrin (PE): CD44–FITC, CD34–PE (Ancell), CD105–PE (eBioscience), CD73–PE, CD90–PE, CD45–PE, CD14–FITC, CD19–PE, and HLA-DR–FITC (BD Pharmingen). PE-conjugated IgG1 or FITC-conjugated IgG2a (BD Pharmingen) was used as an isotype control.

Adipogenic differentiation

WJ-MSCs were induced to differentiate into adipocytes as previously described.[2] Cells were cultured for 21 days in adipogenic differentiation medium consisting of Dulbecco's modified Eagle's medium-low glucose (DMEM-LG; Invitrogen) supplemented with 10% fetal bovine serum (FBS; Sijiqing), 2 mM L-glutamine (Invitrogen), 1% penicillin-streptomycin (Invitrogen), 60 μM indomethacin, 1 μM dexamethasone, 0.5 mM 3-isobutyl-1-methylxanthine (IBMX), and 5 μg/mL insulin solution (Sigma). WJ-MSCs were cultured in the growth medium as a negative control. The generation of neutral lipid vacuoles was visualized by staining with Oil Red O (Sigma).



SUPPLEMENTARY FIG. S1. Analysis of the typical mesenchymal stem cell (MSC) marker expression by flow cytometry. Mesenchymal stem cells from human umbilical cord Wharton's jelly (WJ-MSCs) were stained with phycoerythrin - or fluorescein isothiocyanate-conjugated antibodies. The *open areas* represent antibody isotype controls for background fluorescence, and the *shaded areas* show the expression of the different MSC markers. WJ-MSCs were positive for typical MSC markers CD44, CD73, CD90, and CD105, and negative for control markers CD14, CD19, CD34, CD45, and HLA-DR.

Osteogenic differentiation

WJ-MSCs were induced to differentiate into osteoblasts as previously described.[3] Cells were cultured for 14 or 28 days in osteogenic differentiation medium consisting of DMEM-LG (Invitrogen) supplemented with 10% FBS (Sijiqing), 0.2 mM L-ascorbate (Sigma), 1% penicillin-streptomycin (Invitrogen), 100 mM dexamethasone, and 10 mM β-glycerophosphate (Sigma). WJ-MSCs were cultured in the growth medium as a negative control. Osteogenic differentiation was examined by alkaline phosphatase staining with the alkaline phosphatase kit (Sigma). Mineralization of osteogenic differentiation was assessed by von Kossa staining as previously described.[3]

Chondrogenic differentiation

Chondrogenic differentiation was performed as previously described.[2] WJ-MSCs were cultured for 21 days in chondrogenic differentiation medium containing Dulbecco's modified Eagle's medium-high glucose (Gibco) supplemented with 1% insulin-transferrin-selenium, 1% penicillin-streptomycin (Invitrogen), 0.1 μM dexamethasone, 50 μg/mL ascorbate (Sigma), 40 μg/mL L-proline, 100 μg/mL sodium pyruvate, and 10 ng/mL transforming growth factor-β1 (PeproTech). Cells were cultured in the growth medium as a negative control. Differentiated cells were examined by Alcian blue staining.

Hepatocyte differentiation

Hepatocyte differentiation was performed as previously described.[4] WJ-MSCs were cultured for 21 days in hepatocyte differentiation medium containing Iscove's modified Dulbecco's medium (Invitrogen) supplemented with 1% FBS, 40 ng/mL hepatocyte growth factor, 10 ng/mL fibroblast growth factor-4 (PeproTech), 1 μg/mL amphotericin, and 1% penicillin-streptomycin (Invitrogen). WJ-MSCs were cultured in the growth medium as a negative control.

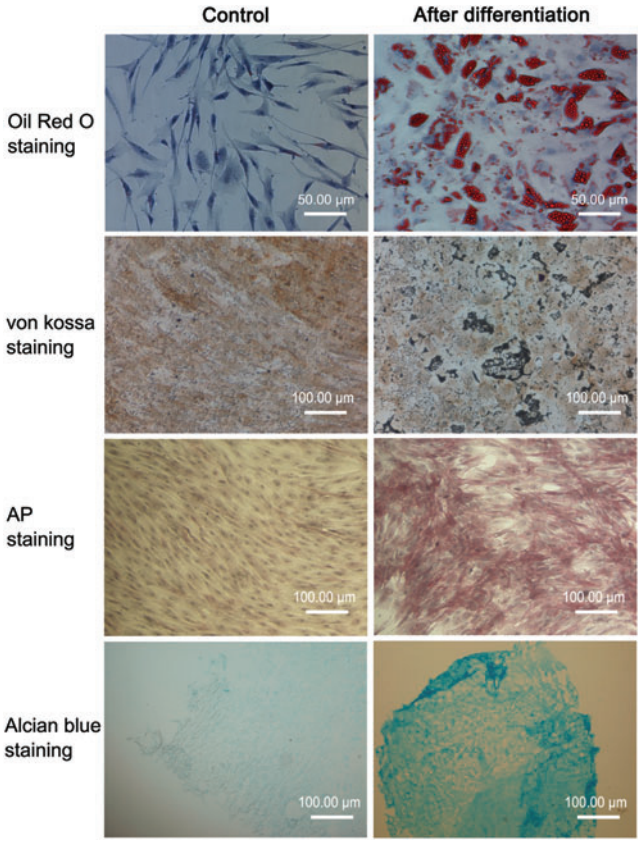
Western blot analysis

WJ-MSCs were cultured for 7, 14, and 21 days in the hepatocyte differentiation medium. Total cellular proteins were transferred to a polyvinylidene fluoride western blotting membrane (Roche Diagnostics Corporation). The membrane was blocked in the blocking solution for 2 h and incubated overnight at 4°C with mouse monoclonal antibodies against albumin (ALB), α-fetoprotein (AFP), and cytokeratin 18 (CK-18). The membrane was incubated for 60 min with horseradish peroxidase-linked goat anti-mouse immunoglobulin G. Protein bands were visualized using BeyoECL Plus (Beyotime). Mouse monoclonal antibody against tubulin was used as a control.

Low-density lipoprotein uptake assay

Low-density lipoprotein (LDL) uptake assay was performed as previously described.[5] After WJ-MSCs were cultured for 21 days in the hepatocyte differentiation medium, cells were incubated in the hepatocyte differentiation medium containing 10 μg/mL of 1,1′-dioctadecyl-1-3,3,3′,3′-tetramethyl-indo-carbocyanine perchlorate conjugated to acetylated-LDL (Molecular Probes) for 24 h at 37°C. Cell nuclei were counterstained with diamidinophenylindole (Beyotime) for 15 min at 37°C, and cells were observed under a fluorescent microscope.

See the main article for the following methods: Immunocytochemistry, periodic acid–Schiff staining for glycogen storage, blood assays, hematoxylin-eosin staining, and Masson staining.

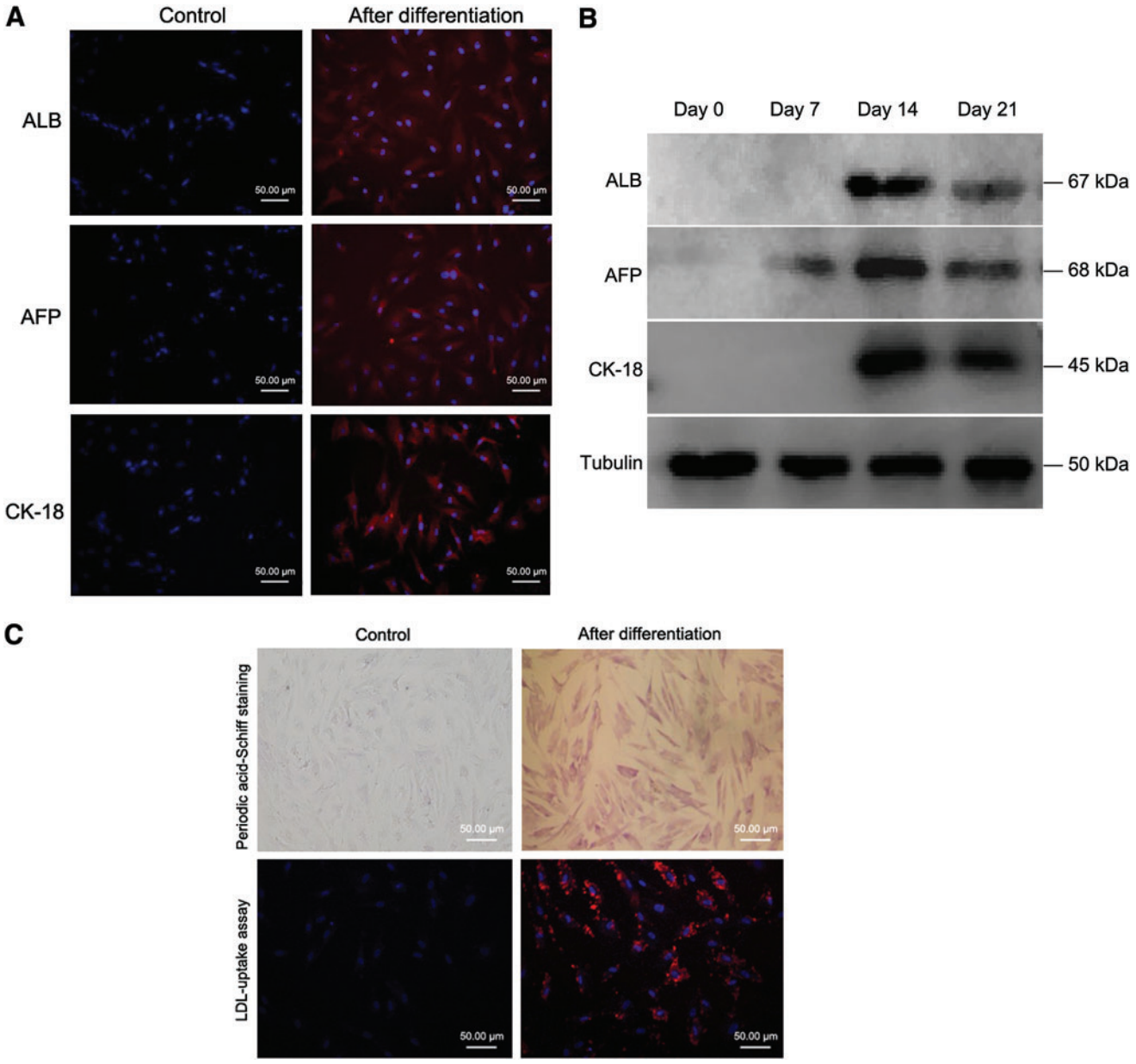


SUPPLEMENTARY FIG. S2. Adipogenic, osteogenic, and chondrogenic differentiation of WJ-MSCs. To confirm WJ-MSCs isolated in this study had the typical multipotent differentiation potential, they were induced to differentiate into adipogenic, osteogenic, and chondrogenic cells. Adipogenic differentiation was visualized by staining with Oil Red O. Osteogenic differentiation was detected by von Kossa staining and AP staining. Chondrogenic differentiation was examined by Alcian blue staining. WJ-MSCs could be induced to differentiate into adipocytes, osteoblasts, and chondrocytes. WJ-MSCs were cultured in the growth medium as controls and did not show any staining.

Results

Analysis of the typical MSC marker expression by flow cytometry

Flow cytometry analysis showed that WJ-MSCs were positive for MSC markers CD44, CD73, CD90, and CD105 and negative for control markers CD14, CD19, CD34, CD45, and HLA-DR as expected (Supplementary Fig. S1). These data confirmed that WJ-MSCs used for this study had the typical MSC marker expression.



SUPPLEMENTARY FIG. S3. Characterization of hepatocyte-like cells. **(A)** Detection of hepatocyte-specific marker expression by immunocytochemistry. Cells became positive for the hepatocyte-specific markers after differentiation. Cells were cultured in the growth medium as negative controls. **(B)** Analysis of the hepatocyte-specific protein expression by western blot analysis. WJ-MSCs cultured in the growth medium were defined as day 0. Tubulin was used as a control. The size of each protein was indicated. Cells expressed the hepatocyte-specific markers after differentiation. **(C)** Periodic acid-Schiff staining and low-density lipoprotein (LDL) uptake assay. Cells were cultured in the growth medium as negative controls. Cells became positive after differentiation.

Multipotent differentiation potential of WJ-MSCs

To confirm the multipotent differentiation potential of WJ-MSCs, they were induced to differentiate into adipogenic, osteogenic, and chondrogenic cells with different differentiation media. The results showed that WJ-MSCs isolated in this study had multipotent differentiation potential (Supplementary Fig. S2).

Detection of hepatocyte-specific marker expression

WJ-MSCs were cultured for 21 days in the hepatocyte differentiation medium, immunocytochemistry showed that they became positive for hepatocyte-specific markers ALB, AFP, and CK-18 (Supplementary Fig. S3A). Cells were cultured in the growth medium as negative controls and did not show any positive expression. Western blot analysis

confirmed these results and showed that ALB and CK-18 had a similar pattern of protein expression, and their expression was detected on day 14 and 21 but not day 7 following hepatocyte differentiation (Supplementary Fig. S3B). AFP expression was first detected on day 7 and day 14 and 21 following hepatocyte induction. The expression of tubulin was used as a control. These results demonstrated that WJ-MSCs used for this study could differentiate into hepatocyte-like cells *in vitro* and suggest that they may also differentiate into hepatocyte-like cells *in vivo*.

Periodic acid–Schiff staining and LDL-uptake assay

After WJ-MSCs were cultured for 21 days in the hepatocyte differentiation medium, cells were positively stained by periodic acid-Schiff for glycogen accumulation and could uptake LDL by LDL-uptake assay (Supplementary Fig. S3C). Cells were cultured in growth medium as negative controls. These results further confirmed that WJ-MSCs used for this study could differentiate into hepatocyte-like cells *in vitro*.

Supplementary References

1. Zhang, Y.N., Lie, P.C., and Wei, X. Differentiation of mesenchymal stromal cells derived from umbilical cord Wharton's jelly into hepatocyte-like cells. Cytotherapy **11,** 548, 2009.
2. Wang, H.S., Hung, S.C., Peng, S.T., Huang, C.C., Wei, H.M., Guo, Y.J., *et al.* Mesenchymal stem cells in the Wharton's jelly of the human umbilical cord. Stem Cells **22,** 1330, 2004.
3. Kern, S., Eichler, H., Stoeve, J., Klüter, H., and Bieback, K. Comparative analysis of mesenchymal stem cells from bone marrow, umbilical cord blood, or adipose tissue. Stem Cells **24,** 1294, 2006.
4. Taléns-Visconti, R., Bonora, A., Jover, R., Mirabet, V., Carbonell, F., Castell, J.V., *et al.* Hepatogenic differentiation of human mesenchymal stem cells from adipose tissue in comparison with bone marrow mesenchymal stem cells. World J Gastroenterol **12,** 5834, 2006.
5. Schwartz, R.E., Reyes, M., Koodie, L., Jiang, Y., Blackstad, M., Lund, T., *et al.* Multipotent adult progenitor cells from bone marrow differentiate into functional hepatocyte-like cells. J Clin Invest **109,** 1291, 2002.