Materials and Methods

The separation of hUC-MSCs

This study has been authorized by the IRB of the authors' affiliated institutions. The study was approved by the Board and Ethics Committee of Weihai Central Hospital Affiliated to Qingdao University. All participants approved the written informed consent. The umbilical cord samples were obtained from the normal term cesarean section neonates. The mother and child with any infectious diseases and/or family genetic diseases were eliminated, and informed consent was permitted from the mother and her family. The fresh umbilical cords were taken about 10 cm aseptically and rinsed away the residual blood with phosphate buffer saline (PBS) (YuanMu Biological Technology, Shanghai, China). After umbilical cords were cut into small pieces of 2 to 3 cm and washed with PBS again, they were cut longitudinally, removed the one umbilical vein and two umbilical arteries, and peeled off the Wharton's jelly. Then, the Wharton's jelly was cut into small tissue pieces and maintained in MDS1601 culture medium (AKSO CELL) including 10% foetal bovine serum (FBS) (Gibco, Rockville, MD, USA), 1% penicillin-streptomycin (HyClone, Logan City, Utah, USA) at 37℃ with 5% CO_2. After 7 days, the Wharton's jelly tissues were discarded and the culture medium was replaced for the first time. Afterwards, the medium was replaced once every 2 days. Cells were passaged until the confluent of cells reached about 80%.

Flow cytometry assay

When the third-generation cells reached approximately 90% confluent, culture medium was removed and PBS was utilized to rinse cells 2 to 3 times. Subsequently, cells were digested with trypsin-EDTA solution, resuspended in PBS and count to 1×10^6/ml. Next, cell suspension was maintained with CD14-APC (MHCD1405), CD29-APC (17-0299-41), CD34-PE (CD34-581-04), CD44-FITC (MHCD4401), CD45-APC (MHCD4505), CD73-FITC (11-0739-42), CD90-APC (A14727), CD105-PE (MHCD10504) and HLA-DR-APC (MHLDR05) (Caltag, Thermo Fisher Scientific, Waltham, MA, USA) in the dark for 30 min. The expression of surface antigens was determined using flow cytometer (B&D SYSTEMS).

Results

The separation and identification of hUC-MSCs

The Wharton's jelly was successfully isolated from the umbilical cord tissue and observed with an inverted microscope after cultivation. The tissues adhered to the wall after 1～2 days of cultivation. Then, some cells could be found with fusiform and spindly morphology after 7 days. About 14 days, the cells reached 80% confluent and formed circinate cell colonies. After 2 passages, the cell growth ability was notably increased, and the cells displayed a uniform long spindle shape, resembling fibroblasts, and grew in a typical spiral arrangement. The morphology of the cells did not alter observably during the progress of proliferation and passage. Flow cytometry analysis showed that hUC-MSCs at passage 3 had a significant expression of CD29, CD44, CD73, CD90 and CD105, and almost did not express CD14, CD34, CD45 and HLA-DR. Thus, these findings indicated that the hUC-MSCs was successfully acquired for further assays.

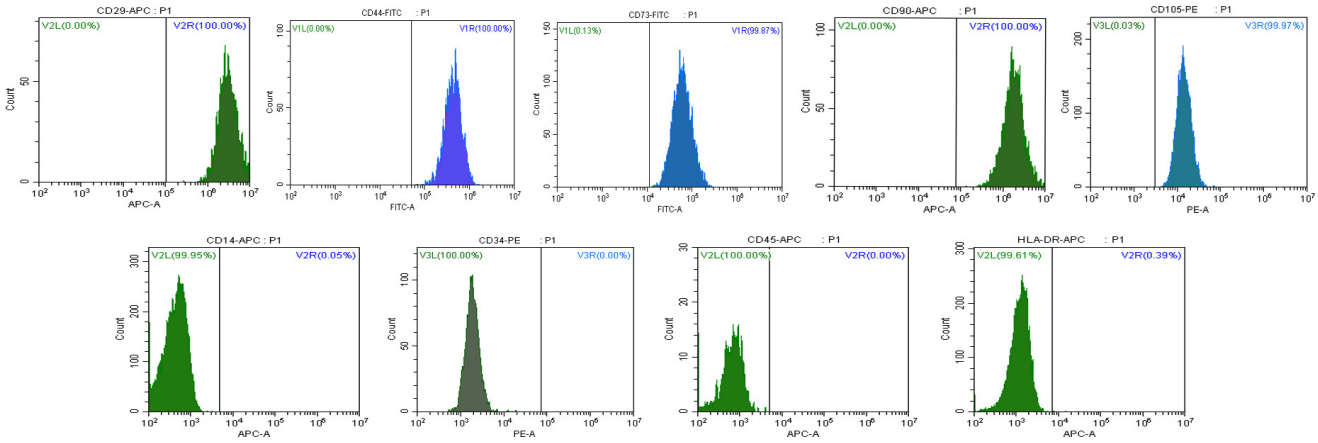

Fig. S1. The separation and identification of hUC-MSCs. Surface markers of hUC-MSCs examined by flow cytometry.