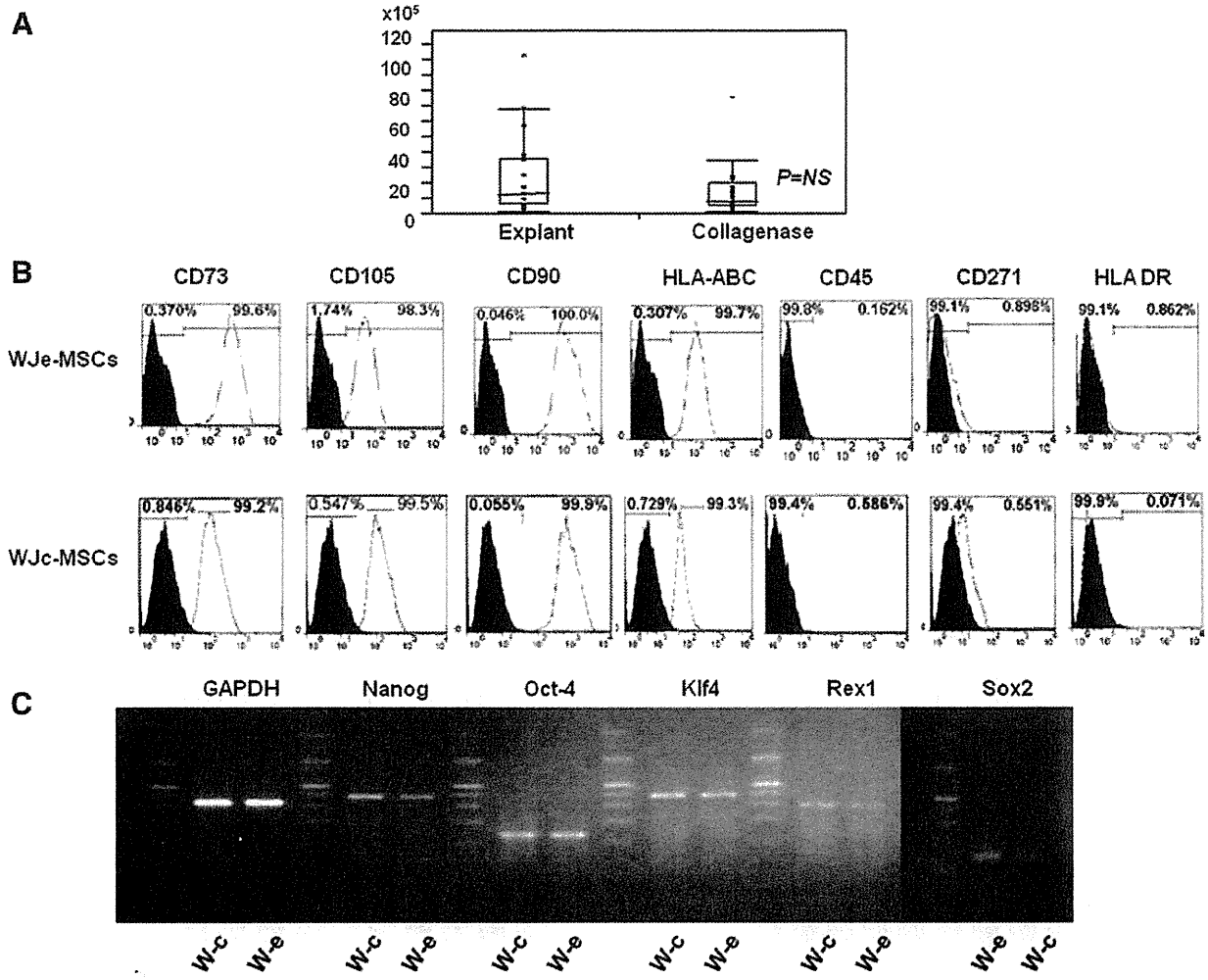

FIG. 2. Characterization of WJ-MSCs collected by the explant and the collagenase-treatment methods. **(A)** Median cell numbers of WJ-MSCs collected by the explant method ($n=23$) and the collagenase-treatment method are shown ($n=20$). **(B)** Both WJe-MSCs and WJc-MSCs were positive for CD73, CD90, CD105, CD271, and HLA class I and negative for CD45 and HLA class II. **(C)** Expression of embryonic stem cell–related markers *Nanog*, *Oct4*, *Klf4*, *Rex1*, and *Sox2* is shown. The data are representative of three individual experiments.

(100 nM/mL) of BMP2 and a longer culture period of 5 weeks ($n=3$; Fig. 5B).

Expression of SSEA4/3 in media with different FBS concentrations

We examined SSEA4/3 expression after 1 week in cultures containing 0.1%, 1%, 10%, and 20% FBS, which contains GSL. The cultured WJe-MSCs were all positive for CD73, and cell proliferation of WJe-MSCs was associated with FBS concentration (Fig. 6A, B). The SSEA4 expression was positively correlated with the FBS concentration (Fig. 6C), whereas SSEA3 expression was negatively correlated (Fig. 6D). The WJe-MSCs were 15.8%±6.2% $SSEA4^+CD73^+$ and 5.8%±1.9% $SSEA3^+CD73^+$ in 0.1% FBS medium, 24.5%±9.8% and 2.7%±1.0% in 1% FBS, 41.5%±13.1% and 0.7%±0.5% in 10% FBS, and 48.3%±12.0% and 0.6%±0.4% in 20% FBS, respectively ($n=3$).

To see that the increase in SSEA4 expression upon increasing FBS concentration is caused by the change in expression alone but not because of increased WJe-MSC proliferation, we analyzed SSEA4 expression associated with growth curve with different FBS concentrations. In consistent with the prior data, SSEA4 expression was correlated with FBS concentration, while SSEA3 was inversely correlated (Fig. 6E–G). The higher FBS concentration accelerated the proliferation of WJe-MSCs with higher expression of SSEA4 (Fig. 6E, F). The incidence of $SSEA4^+$ and $SSEA3^+$ cells was highest on day 3, followed by the decline from days 3 to 7, still during proliferation. The possibility of the substrate shortage for SSEA4 and SSEA3 during culture could be denied, because we replaced the fresh medium on day 4. The data are representative of three individual experiments.

Next, when the sorted $SSEA4^+$ and $SSEA4^-$ WJe-MSCs were cultured in 1%, 5%, and 10% FBS for 1 week, SSEA4

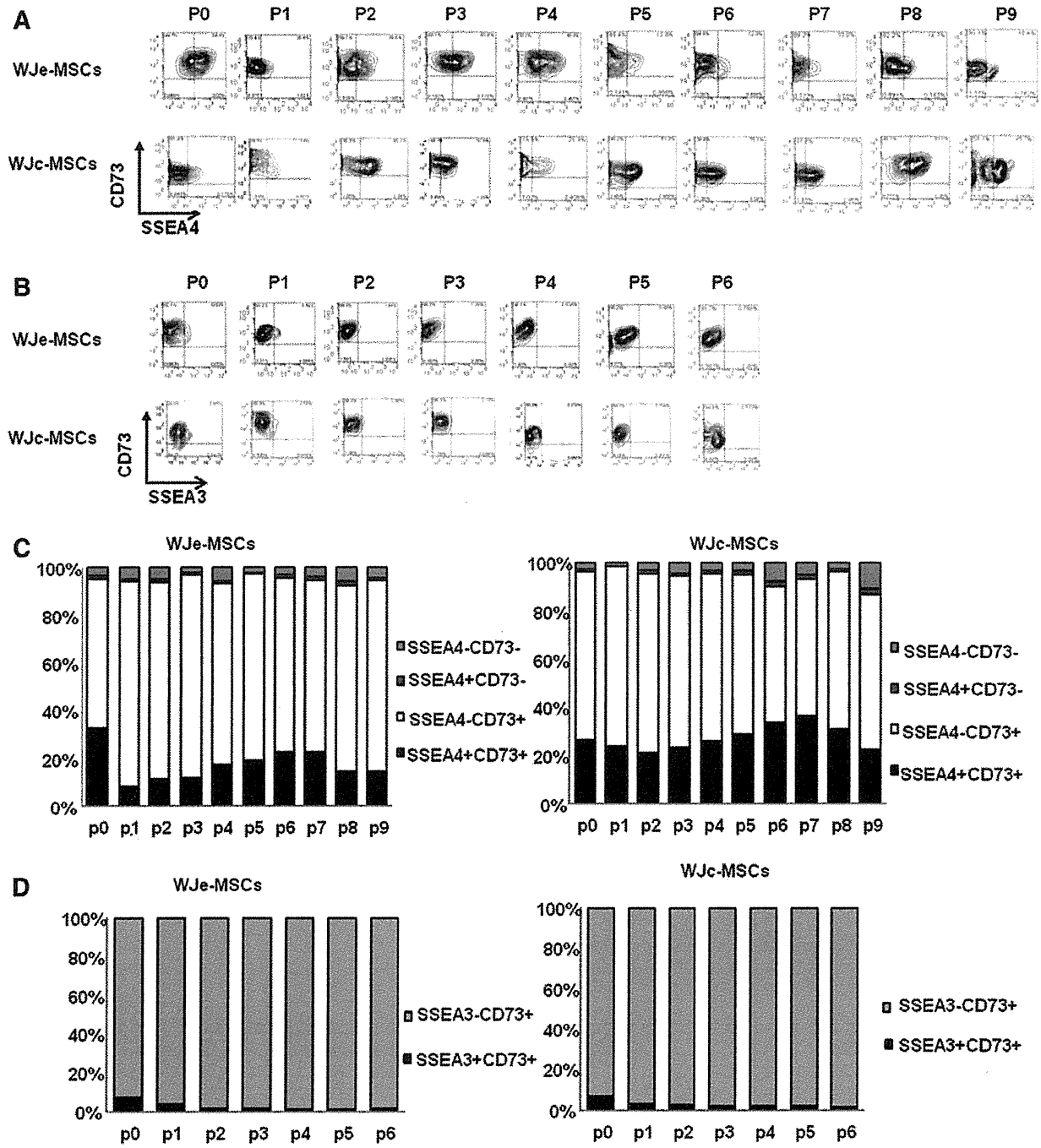

FIG. 3. Stage-specific embryonic antigen-4 expression during passages. **(A)** Stage-specific embryonic antigen 4 (SSEA4) and CD73 expression was monitored in WJ-MSCs collected by the explant (WJe-MSCs) and the collagenase-treatment methods (WJc-MSCs) during P0–P9. **(B)** SSEA3 and CD73 expression was periodically monitored in WJe-MSCs and WJc-MSCs during P0–P6. **(C)** The mean incidence of SSEA4 and CD73 in WJe-MSCs and WJc-MSCs is shown. **(D)** The mean incidence of SSEA3 in $CD73^+$ WJe-MSCs and WJc-MSCs is shown. The data are representative of eight individual experiments.

expression in the sorted $SSEA4^+$ WJe-MSCs was 4.74% and 17.4% in 1% and 10% FBS medium, respectively, whereas SSEA4 expression in the sorted $SSEA4^-$ WJe-MSCs was 2.69% and 5.08% in 1% and 10% FBS, respectively ($n=3$; Fig. 6H, I).

Further, to clarify whether the phenomena of SSEA4 expression influenced by FBS concentration were limited to WJe-MSCs, we performed the same experiments using BM-MSCs derived from frozen-thawed MNCs. As shown in Figure 7A, BM-MNCs before culture included hematopoietic

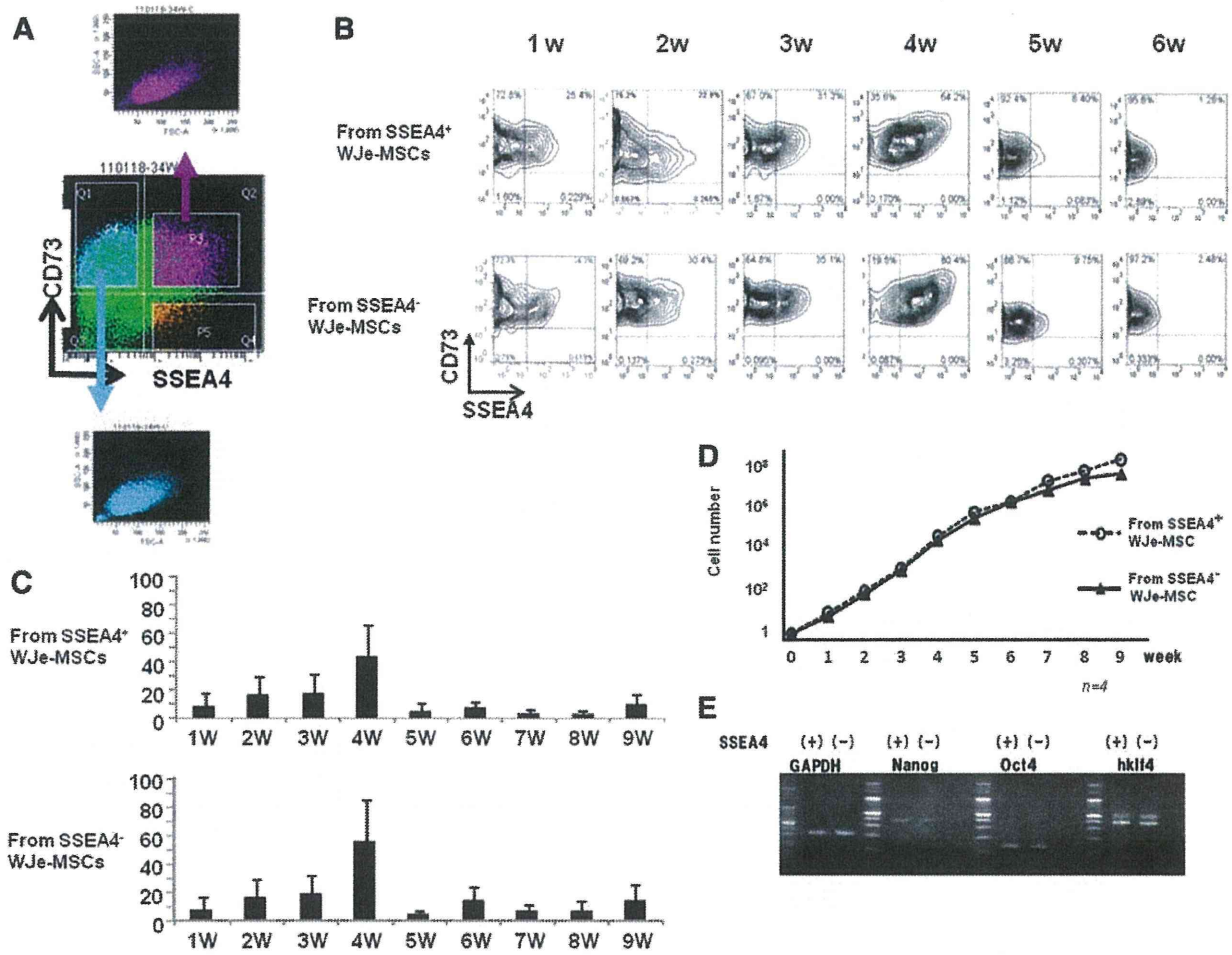

FIG. 4. Sorted SSEA4$^+$ and SSEA4$^-$ WJ-MSCs collected by the explant method. (**A**) SSEA4$^+$CD73$^+$ WJ-MSCs and SSEA4$^-$CD73$^+$ WJe-MSCs were sorted by FACSAria. (**B**) SSEA4 and CD73 expression in sorted SSEA4$^+$ and SSEA4$^-$ WJe-MSCs during passages 1–6 weeks (w) is shown. (**C**) The percentages of SSEA4$^+$ cells in the sorted SSEA4$^+$ and SSEA4$^-$ WJe-MSCs during weeks 1–9 are shown ($n=3$). (**D**) Growth curves of the sorted SSEA4$^+$ (open circles) and SSEA4$^-$ (closed triangles) WJe-MSCs over 9 weeks are shown. (**E**) Gene expression analysis of the sorted SSEA4$^+$ and SSEA4$^-$ WJe-MSCs showed that both were positive for *Oct4*, *Nanog*, and *Klf4*. Color images available online at www.liebertpub.com/tea

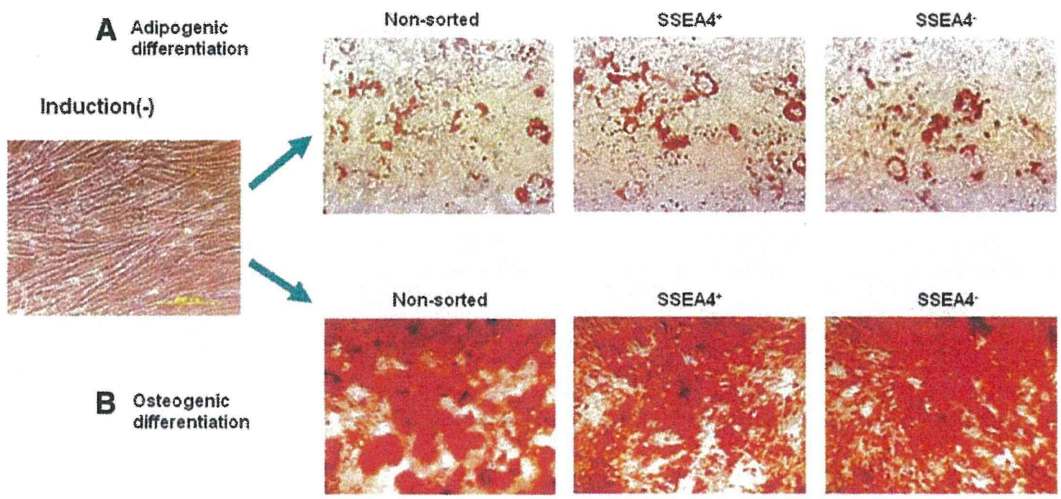

FIG. 5. Adipogenic and osteogenic differentiation of sorted SSEA4$^+$ and SSEA4$^-$ WJ-MSCs. (**A**) Adipogenic differentiation of nonsorted and SSEA4$^+$- and SSEA4$^-$-sorted WJe-MSCs is shown by Red O-stained lipid drops at 3 weeks. (**B**) Osteogenic differentiation of unsorted and SSEA4$^+$- and SSEA4$^-$-sorted WJe-MSCs is shown. Color images available online at www.liebertpub.com/tea

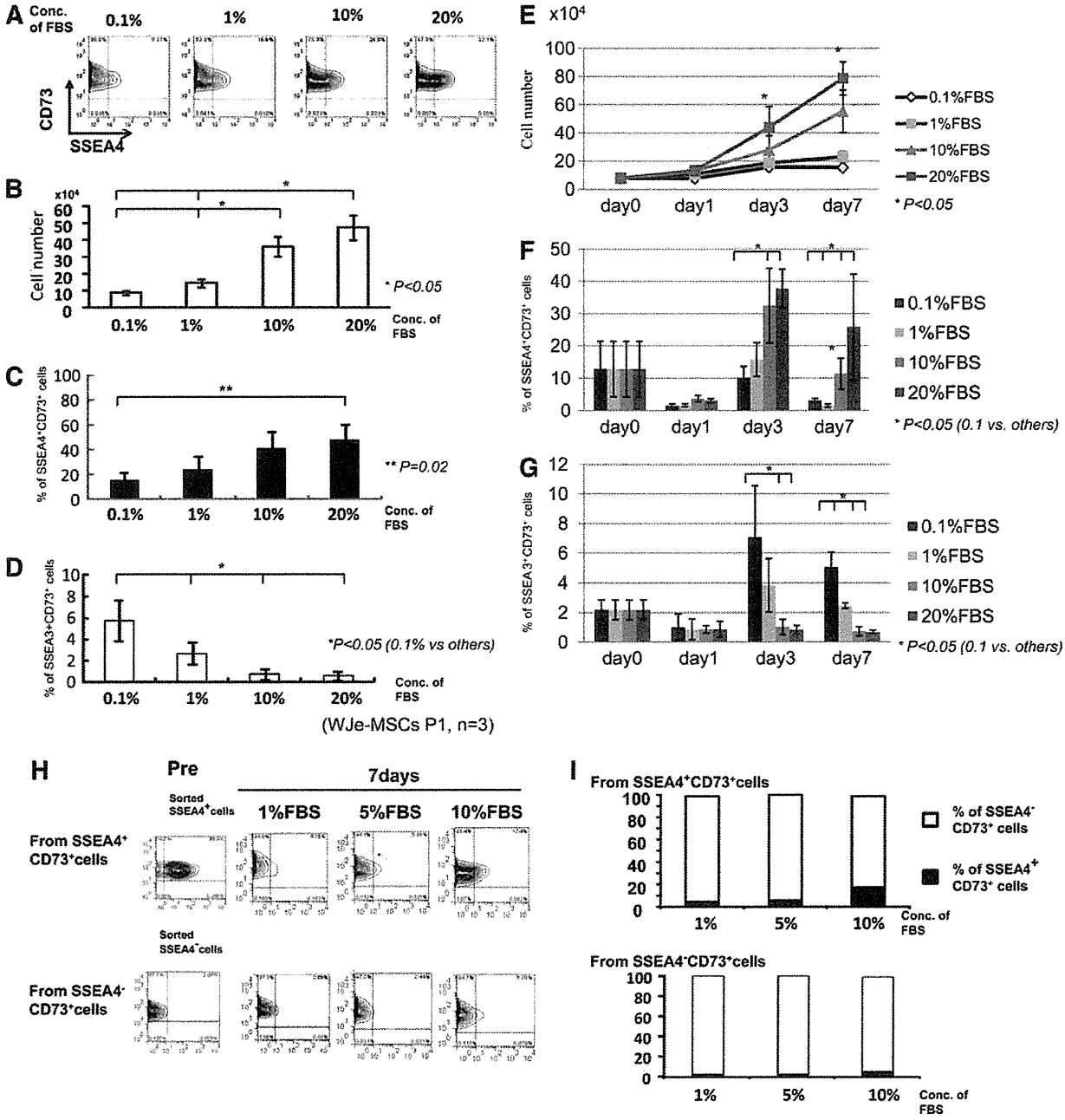

FIG. 6. Influence of fetal bovine serum (FBS) on SSEA4 and SSEA3 expression. **(A)** WJ-MSCs collected by the explant method (WJe-MSCs) were cultured for 1 week in media containing 0.1%, 1%, 10%, and 20% FBS, and SSEA4 expression patterns were analyzed by flow cytometry. The data are representative of three individual experiments. **(B)** WJe-MSC numbers in media with different concentrations of FBS were determined. **(C, D)** Percentages of $SSEA4^+$ and $SSEA3^+$ cells, respectively, among the WJe-MSCs in media with different concentrations of FBS were determined. **(E)** Growth curves of WJe-MSCs with different concentrations of FBS. **(F, G)** Percentage of $SSEA4^+$ and $SSEA3^+$ cells during the proliferation of WJe-MSCs, respectively. **(H, I)** Flow cytometry patterns and percentages of SSEA4 expression in the sorted $SSEA4^+$ and $SSEA4^-$ WJe-MSCs in media with 1%, 5%, and 10% FBS were determined. $^*P<0.05$, $^{**}P=0.02$.

cells with low incidence of MSCs and also low incidence of SSEA4+cells. As expected, the proportion of $CD45^+$ hematopoietic cell expression declined during the passages; instead, $CD45^-$ cells increased (Fig. 7A). The latter cells expressed $CD45^-CD73^+$ MSCs (data not shown). However, in α-MEM with 10% FBS, we could not observe the predominant growth of $SSEA4^+$ cells regardless of CD45 expression (Fig. 7A). Further, in consistent with the results in WJ-MSCs, the SSEA4 expression in BM-MSCs was correlated with the FBS concentration, whereas SSEA3

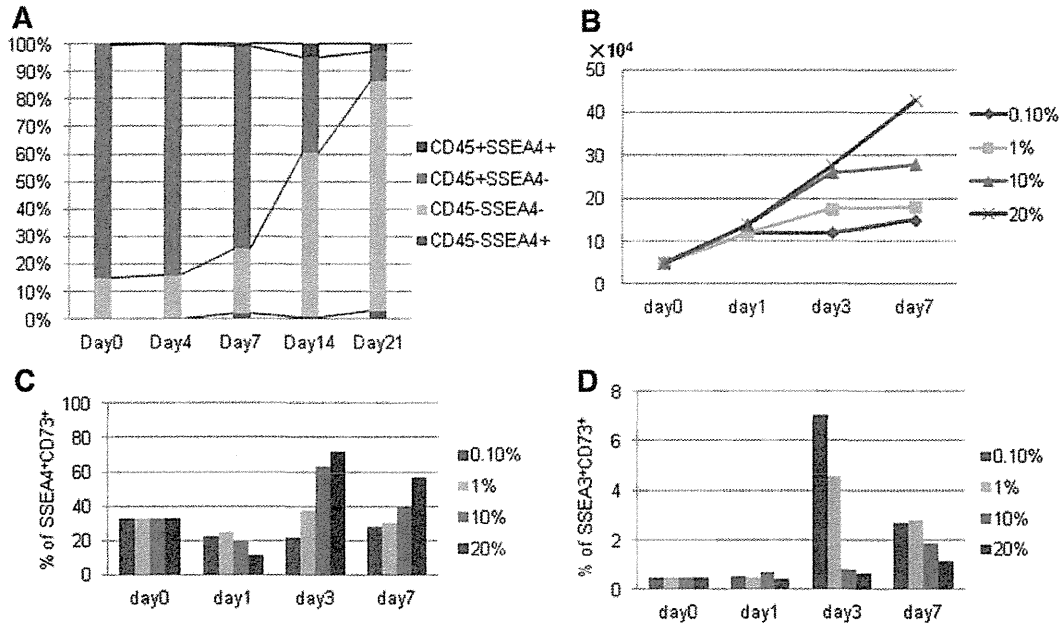

FIG. 7. Influence of FBS on SSEA4 and SSEA3 expression in bone-marrow-derived mesenchymal stem cells. **(A)** Shift of the proportion of $CD45^+$ hematopoietic cells and $CD45^-$ cells with or without SSEA4 expression during the passages. **(B)** Growth curves of BM-MSCs with different concentrations of FBS. **(C, D)** Percentage of $SSEA4^+$ and $SSEA3^+$ cells during the proliferation of BM-MSCs, respectively. The data are representative of two individual experiments.

expression was inversely correlated (Fig. 7B–D). The relationship between the growth curve and SSEA4/3 expression was also consistent with the results in WJ-MSCs (Figs. 6E–G and 7B–D).

Discussion

To gain insight into the role of SSEA3 and SSEA4 in UC WJ-MSCs, we examined the SSEA3 and SSEA4 expression on WJ-MSCs obtained by different methods and compared the differentiation abilities of $SSEA4^+$ and $SSEA4^-$ cells.

First, we compared SSEA3 and SSEA4 expression in WJe-MSCs and WJc-MSCs. The explant method has several advantages. It does not require nonhuman collagenase derived from *Clostridium histolyticum* and saves the time for lysing the tissue with enzyme in the procedure. The disadvantages of the explant method are that collecting adequate cell numbers is dependent on the amount of WJ tissue fragments that attach firmly to the bottom of the dish, which in turn, is dependent on the individual ability. Although the collagenase process from cutting tissue to plating can be unified, we found that some samples are more sensitive to collagenase, resulting in reduced viability. We did not find any significant differences between WJe-MSCs and WJc-MSCs in cell numbers, MSC surface markers as defined by the ISCT, or ES-cell-related gene expression at P0. In addition, the incidence of $SSEA4^+$ and $SSEA3^+$ at P0 was similar between the two types. However, SSEA3 disappeared rapidly in the early culture passages, as described previously.[14,20] It is known that SSEA3 disappears more rapidly from the cell surface compared with SSEA4, if GSL synthesis is blocked by inhibitors, and a similar phenomenon has also been detected during ES cell differentiation.[14,21] Interestingly, the incidence of WJe-MSC $SSEA4^+$ cells was reduced after the first passage, and, by P7, the original levels were recovered. In contrast, the incidence of WJc-MSC $SSEA4^+$ cells was relatively stable until P9. The reason for the depression of SSEA4 expression in WJe-MSCs at P1 is unknown. However, $SSEA4^+$ MSCs in both WJe-MSCs and WJc-MSCs did not proliferate predominantly in our culture medium. As previously reported, osteogenic differentiation was difficult with WJ-MSCs,[8] requiring a longer induction period, additional cytokines such as BMP2, and specific FBS concentrations. However, both sorted $SSEA4^+$ and $SSEA4^-$ WJ-MSCs eventually differentiated into osteocytes and adipocytes in a similar manner, and there were no differences in ES-marker gene expression between the $SSEA4^+$ and $SSEA4^-$ MSCs. Interestingly, $SSEA4^+$ cells appeared even from the $SSEA4^-$ MSCs, and the incidence of $SSEA4^+$ cells derived from the $SSEA4^-$ MSCs demonstrated a similar transition pattern as those derived from the $SSEA4^+$ MSCs. This result suggested that the culture medium may have been the source of SSEA4 antigens.

The role of SSEA3 and SSEA4 in MSCs remains controversial. Rostovskaya *et al.* also suggested SSEA4-marked adipogenic progenitor lacking osteogenic capacity.[22] In ES cell study, Ramirez *et al.* demonstrated that both SSEA3 and SSEA4 are markers of immature ES cells, but particularly SSEA3 together with OCT4 and TRA-1–60 were good tracers for validating pluripotent stem cells, whereas SSEA4 was expressed for long during the differentiation of ES cells.[20] Gang *et al.* reported that $SSEA4^+$ cells proliferated predominantly when the culture was initiated from primary BM cells, which were mostly hematopoietic cells.[11] But in our culture condition, the incidence of $SSEA4^+$ cells, which were characterized as MSCs, was not increased dramatically

as reported by Gang's group. Their results might be induced by the special cocktail of the medium, consisted of MCDB-201, 10% FBS, ITS, linoleic acid–bovine serum albumin, dexamethasone, ascorbic acid, hPDGF-BB, and hEGF, followed by the medium with relatively high FBS concentration. In other articles, Schrobback *et al.* assessed the SSEA4 in human articular chondrocytes, osteoblasts, and BM-derived MSCs and characterized their differentiation potential. But their results showed that SSEA4 levels in these cells were not unrelated to the cells' chondrogenic and osteogenic and proliferation potentials *in vitro*.[23] Suila *et al.* reported that SSEA4, and not SSEA3, was expressed on the surface of cord-blood-derived MSCs, whereas SSEA3 was expressed at very low levels in cord blood hematopoietic stem cells.[24] They also suggested that FBS contains detectable amounts of globoseries GSLs and showed that the SSEA3 was influenced and upregulated by culturing with FBS overnight, even though they did not demonstrate an influence on SSEA4 expression. In our study, we demonstrated that SSEA4 expression significantly correlated with FBS concentration, whereas SSEA3 appeared to be negatively correlated with FBS concentration. The possibility that FBS stimulated the proliferation of WJe-MSCs, resulting in the increase of SSEA4 expression, cannot be denied completely. But the fact that the SSEA4 expression was declined during the proliferation of WJe-MSCs and BM-MSCs in each medium did not support this hypothesis. Regardless of cell growth or cell concentration, consistently, the SSEA4 expression was clearly associated with FBS concentration not only in WJ-MSCs but also in BM-MSCs. Reversely to the SSEA4 expression, SSEA3 expression appeared to be negatively correlated with FBS concentration. The reason why the data of SSEA3 elevation upon the higher FBS concentration are not coincident with Suila's data remained unresolved. We add a partial speculation that SSEA4 is derived from SSEA3; thus, the increase of SSEA4 means the waste of SSEA3, or MSCs with high concentration of FBS are differentiated accompanied with decrease of SSEA3. But we need the scrupulous attention to analyze SSEA3 by FCM in various FBS concentrations. We also found that SSEA4 could be induced from pure $SSEA4^-$ WJ-MSCs. This suggests the presence of substrate for SSEA4 in $SSEA4^-$ cells and also the influence of FBS on the SSEA4 expression in $SSEA4^-$ cells. Brimble *et al.* reported that the depletion of these two molecules by the addition of GSL synthesis inhibitors apparently did not affect the ES cell pluripotency.[14] In conclusion, these results indicate that SSEA4 may display altered expression profiles in response to culture medium including FBS and may not be an essential marker of WJ-MSC pluripotency.

Acknowledgments

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Disclosure Statement

The authors have no conflicts of interest.

References

1. Ishige, I., Nagamura-Inoue, T., Honda, M.J., Harnprasopwat, R., Kido, M., Sugimoto, M., *et al.* Comparison of mesenchymal stem cells derived from arterial, venous, and Wharton's jelly explants of human umbilical cord. Int J Hematol **90,** 261, 2009.
2. Kode, J.A., Mukherjee, S., Joglekar, M.V., and Hardikar, A.A. Mesenchymal stem cells: immunobiology and role in immunomodulation and tissue regeneration. Cytotherapy **11,** 377, 2009.
3. Zeddou, M., Briquet, A., Relic, B., Josse, C., Malaise, M.G., Gothot, A., *et al.* The umbilical cord matrix is a better source of mesenchymal stem cells (MSC) than the umbilical cord blood. Cell Biol Int **34,** 693, 2010.
4. Horwitz, E.M., Le Blanc, K., Dominici, M., Mueller, I., Slaper-Cortenbach, I., Marini, F.C., *et al.* Clarification of the nomenclature for MSC: The International Society for Cellular Therapy position statement. Cytotherapy **7,** 393, 2005.
5. Dominici, M., Le Blanc, K., Mueller, I., Slaper-Cortenbach, I., Marini, F., Krause, D., *et al.* Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy **8,** 315, 2006.
6. Anzalone, R., Iacono, M.L., Corrao, S., Magno, F., Loria, T., Cappello, F., *et al.* New emerging potentials for human Wharton's jelly mesenchymal stem cells: immunological features and hepatocyte-like differentiative capacity. Stem Cells Dev **19,** 423, 2010.
7. Carvalho, M.M., Teixeira, F.G., Reis, R.L., Sousa, N., and Salgado, A.J. Mesenchymal stem cells in the umbilical cord: phenotypic characterization, secretome and applications in central nervous system regenerative medicine. Curr Stem Cell Res Ther **6,** 221, 2011.
8. Hsieh, J.Y., Fu, Y.S., Chang, S.J., Tsuang, Y.H., and Wang, H.W. Functional module analysis reveals differential osteogenic and stemness potentials in human mesenchymal stem cells from bone marrow and Wharton's jelly of umbilical cord. Stem Cells Dev **19,** 1895, 2010.
9. Fong, C.Y., Chak, L.L., Biswas, A., Tan, J.H., Gauthaman, K., Chan, W.K., *et al.* Human Wharton's jelly stem cells have unique transcriptome profiles compared to human embryonic stem cells and other mesenchymal stem cells. Stem Cell Rev **7,** 1, 2011.
10. Kannagi, R., Cochran, N.A., Ishigami, F., Hakomori, S., Andrews, P.W., Knowles, B.B., *et al.* Stage-specific embryonic antigens (SSEA-3 and -4) are epitopes of a unique globo-series ganglioside isolated from human teratocarcinoma cells. EMBO J **2,** 2355, 1983.
11. Gang, E.J., Bosnakovski, D., Figueiredo, C.A., Visser, J.W., and Perlingeiro, R.C. SSEA-4 identifies mesenchymal stem cells from bone marrow. Blood **109,** 1743, 2007.
12. Kuroda, Y., Kitada, M., Wakao, S., Nishikawa, K., Tanimura, Y., Makinoshima, H., *et al.* Unique multipotent cells in adult human mesenchymal cell populations. Proc Natl Acad Sci USA **107,** 8639, 2010.
13. Wakao, S., Kitada, M., Kuroda, Y., and Dezawa, M. Isolation of adult human pluripotent stem cells from mesenchymal cell populations and their application to liver damages. Methods Mol Biol **826,** 89, 2012.
14. Brimble, S.N. Sherrer, E.S., Uhl, E.W., Wang, E., Kelly, S., Merrill, A.H. Jr., Robins, A.J., and Schulz, T.C. The cell surface glycosphingolipids SSEA-3 and SSEA-4 are not essential for human ESC pluripotency. Stem Cells **25,** 54, 2007.

15. Tong, C.K., Vellasamy, S., Tan, B.C., Abdullah, M., Vidyadaran, S., Seow, H.F., *et al.* Generation of mesenchymal stem cell from human umbilical cord tissue using a combination enzymatic and mechanical disassociation method. Cell Biol Int **35,** 221, 2011.
16. Lee, K.S., Nah, J.J., Lee, B.C., Lee, H.T., Lee, H.S., So, B.J., *et al.* Maintenance and characterization of multipotent mesenchymal stem cells isolated from canine umbilical cord matrix by collagenase digestion. Res Vet Sci **94,** 144, 2013.
17. Takahashi, K., Tanabe, K., Ohnuki, M., Narita, M., Ichisaka, T., Tomoda, K., *et al.* Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell **131,** 861, 2007.
18. Wulsten, D., Glatt, V., Ellinghaus, A., Schmidt-Bleek, K., Petersen, A., Schell, H., *et al.* Time kinetics of bone defect healing in response to BMP-2 and GDF-5 characterised by *in vivo* biomechanics. Eur Cells Mater **21,** 177, 2011.
19. Agata, H., Asahina, I., Watanabe, N., Ishii, Y., Kubo, N., Ohshima, S., *et al.* Characteristic change and loss of *in vivo* osteogenic abilities of human bone marrow stromal cells during passage. Tissue Eng Part A **16,** 663, 2010.
20. Ramirez, J.M., Gerbal-Chaloin, S., Milhavet, O., Qiang, B., Becker, F., Assou, S., *et al.* Brief report: benchmarking human pluripotent stem cell markers during differentiation into the three germ layers unveils a striking heterogeneity: all markers are not equal. Stem Cells **29,** 1469, 2011.
21. Draper, J.S., Pigott, C., Thomson, J.A., and Andrews, P.W. Surface antigens of human embryonic stem cells: changes upon differentiation in culture. J Anat **200,** 249, 2002.
22. Rostovskaya, M., and Anastassiadis, K. Differential expression of surface markers in mouse bone marrow mesenchymal stromal cell subpopulations with distinct lineage commitment. PloS One **7,** e51221, 2012.
23. Schrobback, K., Wrobel, J., Hutmacher, D.W., Woodfield, T.B., and Klein, T.J. Stage-specific embryonic antigen-4 is not a marker for chondrogenic and osteogenic potential in cultured chondrocytes and mesenchymal progenitor cells. Tissue Eng Part A **19,** 1316, 2013.
24. Suila, H., Pitkanen, V., Hirvonen, T., Heiskanen, A., Anderson, H., Laitinen, A., *et al.* Are globoseries glycosphingolipids SSEA-3 and -4 markers for stem cells derived from human umbilical cord blood? J Mol Cell Biol **3,** 99, 2011.

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LETTER TO THE EDITOR

Pretransplant hyperferritinemia has no effect on the outcome of myeloablative cord blood transplantation for acute leukemia and myelodysplastic syndrome

Takaaki Konuma · Seiko Kato · Maki Oiwa-Monna · Arinobu Tojo · Satoshi Takahashi

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Dear Editor,

Pretransplant hyperferritinemia has been associated with an increased incidence of morbidity and mortality following allogeneic hematopoietic stem cell transplantation (allo-HSCT) after myeloablative or reduced-intensity conditioning [1–3]. Its effect on the outcome of myeloablative cord blood transplantation (CBT) has yet to be clarified. In this study, we retrospectively analyzed whether hyperferritinemia affected the outcome of myeloablative CBT.

Pretransplant serum ferritin level measured within 100 days preceding single-unit CBT for adult patients with acute leukemia or myelodysplastic syndrome (MDS) in our institute between the year 2001 and 2013 was available for 133 patients in this study. All patients received 12 Gy total body irradiation-based myeloablative conditioning regimens and cyclosporine and short-term methotrexate as a graft versus host disease (GVHD) prophylaxis. Cord blood units were selected as reported previously [4, 5]. In multivariate analysis, the following variables were considered: age (<45 vs. ≥45 years), cytomegalovirus serostatus (negative vs. positive), disease status at CBT (standard risk vs. high risk), cord blood nucleated cell count (<2.5 vs. $\geq 2.5 \times 10^7$/kg), cord blood CD34+ cells (<1 vs. $\geq 1 \times 10^5$/kg), HLA disparities based on antigen level HLA-A and -B and allele level HLA-DRB1 (≤2 vs. ≥3), and pretransplant ferritin level (<1,000 vs. ≥1,000 ng/ml). All statistical analyses were performed with EZR, a graphical user interface for R 2.13.0 [6]. $P<0.05$ was considered significant. Analysis of data was performed on August 2013.

The median pretransplant serum ferritin level was 751(range, 58–6,285) ng/ml, the median age was 40(range, 16–55) years, the median number of nucleated cells was 2.52(range, 1.32–5.69)$\times 10^7$/kg, and the median number of CD34+ cells was 0.91(range, 0.28–7.75)$\times 10^5$/kg. Disease types were acute myelogenous leukemia in 74 patients, acute lymphoblastic leukemia in 45, and MDS in 25. The median follow-up of surviving patients was 42(range, 3–103) months after CBT. In univariate and multivariate analysis, we found no impact of hyperferritinemia on overall survival (OS), relapse, transplant-related mortality (TRM), grades II–IV acute GVHD, extensive chronic GVHD, and neutrophil engraftment (Table 1). We also analyzed the effect of hyperferritinemia using different ferritin threshold (500, 1,500, 2,000 ng/ml). But, we were unable to find any impact of hyperferritinemia on outcomes (data not shown).

The effect of hyperferritinemia on outcome might differ depending on the kinds of stem cell sources in allo-HSCT. It has been reported that hyperferritinemia was associated with inferior OS in CBT recipients [7]. However, the cumulative incidence of TRM was very low in our entire cohort, which might have contributed to hyperferritinemia not being shown to influence outcome in our study.

Ferritin is not only a marker for iron overload but also an inflammatory indicator. Moreover, iron overload correlates better with magnetic resonance imaging-measured liver iron content (LIC) than hyperferritinemia. Recently, prospective studies demonstrated that LIC did not affect outcomes of allo-HSCT [8, 9], suggesting that it is unclear whether iron overload

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Table 1 Univariate and multivariate analysis of pretransplant ferritin levels on outcomes of myeloablative CBT

		Univariate analysis[b]		Multivariate analysis[c]	
	Ferritin[a]	% (95 % CI)	*P*	HR (95 % CI)	*P*
Overall survival					
	<1,000 ng/ml	77 (66–85) at 3 years	0.71	1	Reference
	≥1,000 ng/ml	78 (62–87) at 3 years		0.77 (0.37–1.60)	0.49
Relapse					
	<1,000 ng/ml	20 (12–30) at 3 years	0.67	1	Reference
	≥1,000 ng/ml	17 (8–29) at 3 years		0.76 (0.33–1.75)	0.52
Transplant-related mortality					
	<1,000 ng/ml	8 (3–15) at 3 years	0.70	1	Reference
	≥1,000 ng/ml	8 (2–19) at 3 years		0.68 (0.23–2.04)	0.50
Grades II–IV acute GVHD					
	<1,000 ng/ml	70 (59–78) at 100 days	0.34	1	Reference
	≥1,000 ng/ml	63 (47–75) at 100 days		0.79 (0.48–1.29)	0.36
Extensive chronic GVHD					
	<1,000 ng/ml	20 (12–29) at 3 years	0.42	1	Reference
	≥1,000 ng/ml	28 (16–42) at 3 years		1.07 (0.54–2.14)	0.83
Neutrophil engraftment					
	<1,000 ng/ml	92 (82–96) at 60 days	0.28	1	Reference
	≥1,000 ng/ml	95 (81–99) at 60 days		1.13 (0.79–1.62)	0.47

GVHD graft-versus-host disease, *CI* confidence interval, *HR* hazard ratio

[a] Among the 133 patients, pretransplant serum ferritin level was categorized as ≥1,000 ng/ml (n=49) or <1,000 ng/ml (n=84)

[b] The probability of overall survival was estimated according to the Kaplan–Meier method, and the groups were compared using the log-rank test. The probabilities of the others were estimated based on a cumulative incidence method to accommodate competing risks

[c] Multivariate analysis was performed with a Cox proportional hazard model adjusted for overall survival, and Fine and Gray proportional hazards model for the others

affects outcomes of allo-HSCT. Although hyperferritinemia was not associated with adverse outcomes after CBT in this study, further studies are warranted to evaluate the effect of iron overload on the outcome of CBT for acute leukemia and MDS.

Conflict of interest The authors have no conflicts of interest.

References

1. Armand P, Kim HT, Cutler CS, Ho VT, Koreth J, Alyea EP, Soiffer RJ, Antin JH (2007) Prognostic impact of elevated pretransplantation serum ferritin in patients undergoing myeloablative stem cell transplantation. Blood 109(10):4586–4588
2. Majhail NS, Lazarus HM, Burns LJ (2008) Iron overload in hematopoietic cell transplantation. Bone Marrow Transplant 41(12):997–1003
3. Sivgin S, Eser B (2013) The management of iron overload in allogeneic hematopoietic stem cell transplant (alloHSCT) recipients: where do we stand? Ann Hematol 92(5):577–586
4. Takahashi S, Iseki T, Ooi J, Tomonari A, Takasugi K, Shimohakamada Y, Yamada T, Uchimaru K, Tojo A, Shirafuji N, Kodo H, Tani K, Takahashi T, Yamaguchi T, Asano S (2004) Single-institute comparative analysis of unrelated bone marrow transplantation and cord blood transplantation for adult patients with hematologic malignancies. Blood 104(12):3813–3820
5. Takahashi S, Ooi J, Tomonari A, Konuma T, Tsukada N, Oiwa-Monna M, Fukuno K, Uchiyama M, Takasugi K, Iseki T, Tojo A, Yamaguchi T, Asano S (2007) Comparative single-institute analysis of cord blood transplantation from unrelated donors with bone marrow or peripheral blood stem-cell transplants from related donors in adult patients with hematologic malignancies after myeloablative conditioning regimen. Blood 109(3):1322–1330
6. Kanda Y (2013) Investigation of the freely available easy-to-use software 'EZR' for medical statistics. Bone Marrow Transplant 48(3):452–458
7. Matsumoto K, Yamamoto W, Ogusa E, Sugimoto E, Maruta A, Ishigatsubo Y, Kanamori H (2013) Impact of pretransplant serum ferritin on outcome in adult patients receiving cord blood transplant for acute leukemia. Leuk Lymphoma
8. Armand P, Sainvil MM, Kim HT, Rhodes J, Cutler C, Ho VT, Koreth J, Alyea EP, Neufeld EJ, Kwong RY, Soiffer RJ, Antin JH (2012) Does iron overload really matter in stem cell transplantation? Am J Hematol 87(6):569–572
9. Trottier BJ, Burns LJ, Defor TE, Cooley S, Majhail NS (2013) Association of iron overload with allogeneic hematopoietic cell transplantation outcomes: a prospective cohort study using R2-MRI-measured liver iron content. Blood 122(9):1678–1684

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TOPIC HIGHLIGHT

WJSC 6th Anniversary Special Issues (2): Mesenchymal stem cells

Umbilical cord-derived mesenchymal stem cells: Their advantages and potential clinical utility

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Abstract

Human umbilical cord (UC) is a promising source of mesenchymal stem cells (MSCs). Apart from their prominent advantages, such as a painless collection procedure and faster self-renewal, UC-MSCs have shown the ability to differentiate into three germ layers, to accumulate in damaged tissue or inflamed regions, to promote tissue repair, and to modulate immune response. There are diverse protocols and culture methods for the isolation of MSCs from the various compartments of UC, such as Wharton's jelly, vein, arteries, UC lining and subamnion and perivascular regions. In this review, we give a brief introduction to various compartments of UC as a source of MSCs and emphasize the potential clinical utility of UC-MSCs for regenerative medicine and immunotherapy.

Key words: Umbilical cord; Mesenchymal stem cells; Wharton's Jelly; Multipotency; Immunotherapy

Core tip: Human umbilical cord (UC) is a promising source of mesenchymal stem cells (MSCs). UC-MSCs have shown the ability of faster self-renewal and to differentiate into three germ layers, to accumulate in damaged tissue or inflamed regions, to promote tissue repair, and to modulate immune response. There are diverse protocols and culture methods for the isolation of MSCs from the various compartments of UC, such as Wharton's jelly, vein, arteries, UC lining membrane and subamnion and perivascular regions. In this review, we introduce various compartments of UC and discuss the potential clinical utility of UC-MSCs for regenerative medicine and immunotherapy.

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INTRODUCTION

Mesenchymal stem cells (MSCs) originate in the human embryo and are considered multipotent stem cells. MSCs are a heterogeneous subset of stromal stem cells, which can be isolated from the bone marrow[1], mobilized peripheral blood[2], cord blood[3], umbilical cord (UC)[4,5], placenta[6], adipose tissue[7], dental pulp[8], and even the fetal liver[9] and lungs[10]. UC contains two umbilical arteries (UCAs) and one umbilical vein (UCV), both embedded within a specific mucous connective tissue, known as Wharton's jelly (WJ), which is covered by amniotic epithelium (Figure 1). UC is considered medical waste and the collection of UC-MSCs is noninvasive; furthermore, the access to UC-MSCs has not been encumbered with ethical problems. UC-MSCs, similarly to MSCs derived