CD29 of human umbilical cord mesenchymal stem cells is required for expansion of CD34⁺ cells

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Abstract

Objectives: Human umbilical cord mesenchymal stem cells (hUCMSCs) play a critical role in expanding haematopoietic stem cells (HSCs) by providing the essential microenvironment for haematopoiesis. In this study, we sought to investigate whether CD29 of hUCMSCs would play a key role in the ability of hUCMSCs to help expand HSCs *in vivo* and *in vitro*.

Material and methods: To investigate whether CD29 of hUCMSCs would play a key role for the ability of hUCMSCs to expand HSCs, soluble anti-CD29 antibody was added to co-cultures of hUCMSCs and cord blood (CB) CD34⁺ cells. It significantly blocked expansion of CB CD34⁺ cells induced by hUCMSCs. Using CD29-deficient hUCMSCs models, long-term culture-initiating cell and non-obese diabetic/severe combined immunodeficient disease mouse repopulating cell assay, revealed that CB CD34⁺ cells co-cultured with CD29-deficient hUCMSCs only retained the capacity of multipotent differentiation for 5 weeks at the most.

Results: Soluble anti-CD29 antibody significantly blocked expansion of CB CD34⁺ cells induced by hUCMSCs. CB CD34⁺ cells co-cultured with CD29-deficient hUCMSCs only retained the capacity of multipotent differentiation for 5 weeks at the most.

Conclusions: CB CD34⁺ cells co-cultured with CD29-deficient hUCMSCs gave rise to all major

haematopoietic lineages, but failed to engraft long term.

Introduction

Haematopoietic stem cells (HSCs) have extensive regenerative potential which makes them attractive targets for cell and genetic therapies; however, their rarity limits their use for therapeutic application. Large *ex vivo* HSC expansion has been one of the major goals of stem cell research (1–4). Any expansion protocol must attempt to simulate (as close as possible) *in vivo* haematopoiesis, to maintain stemness properties of the HSCs (4–6).

Human mesenchymal stem cells (MSCs) play a critical role in providing the essential microenvironment for haematopoiesis, which has been successfully used *in vitro* as a scaffold for stromal support and expansion of HSCs *via* cell/cell contact (7–11). Biological interest in MSCs, first described by Friedenstein *et al.* (12), has risen dramatically over the last decade. Although bone marrow (BM) remains a major source of MSCs for most investigations, and primary marrow stromal cells are able to support HSC expansion *in vitro*, it has been difficult to isolate adequate primary BM stromal cells for expansion (13,14). Umbilical cord (UC), rich in MSCs, and UC-derived MSCs (UCMSCs) have been shown to be easy to isolate and culture (15,16). Increasing amounts of data have shown that adhesion and direct cell/cell contact between UCMSC and feeder layers supports *ex vivo* expansion, migratory potential and stemness of HSCs (17,18). Although beneficial effects of human UC mesenchymal stem cells (hUCMSCs) on their supportive role in haematopoiesis is known, molecular regulation of interaction between MSCs and HSCs up to now still needed to be elucidated.

CD29, a binding subunit of the $\beta 1$ integrin family receptors, binds various types of ligand such as vascular

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adhesion molecule (VCAM)-1 and extracellular matrix proteins, produced by many stromal cells, and mediates niche interactions (11,19). To investigate molecular regulation of the supportive role of hUCMSCs in haematopoiesis, we formed the hypothesis that CD29 would play a key role in the ability of hUCMSCs to support it, as it mediates niche interactions and is highly expressed by hUCMSCs (10,20,21). To test the hypothesis, first we proved that CD29 was important for the ability of hUCMSCs to support haematopoiesis, by the addition of soluble anti-CD29 antibody to co-cultures of hUCMSCs and CB CD34⁺ cells. Using CD29-deficient hUCMSCs models, long-term culture-initiating cell (LTC-IC) and non-obese diabetic/severe combined immunodeficient disease (NOD/SCID) mouse repopulating cell (SRC) assay revealed that CB CD34⁺ cells co-cultured with CD29-deficient hUCMSCs only retained the capacity of multipotent differentiation for 5 weeks at the most. CB CD34⁺ cells co-cultured with CD29-deficient hUCMSCs gave rise to all major haematopoietic lineages, but failed to engraft long term. CD29-deficient hUCMSCs may interact more loosely with CB CD34⁺ cells, which would promote efficient transition from long-term to short-term HSCs, then speed up efficient and continuous differentiation of HSCs.

In addition to being important for mediating HSC-niche interactions, our data raise the possibility that CD29 in hUCMSCs may also be necessary for the ability of hUCMSCs to expand CB CD34⁺ cells.

Materials and methods

In this study, experimental protocols concerning humans were approved by the Ethics Committee of Peking University. Before experiments, subjects were informed of the objectives, requirements and procedures of the experiments. All subjects gave informed written consent to participate in the study.

Experimental protocols concerning animals had been approved by the Institutional Authority for Laboratory Animal Care, of Peking University.

Isolation and culture of hUCMSCs and cord blood (CB) CD34⁺ cells

After washing in Hanks balanced salt solution to remove contaminating blood, UCs were cut into 1 cm pieces, and vessels were removed to avoid endothelial cell contamination. Tissue pieces were placed in six-well plates for culture expansion in low-glucose Dulbecco's modified Eagle's medium (L-DMEM) (Hyclone, Logan, Utah, USA) supplemented with 10% foetal bovine serum (FBS). Cultures were maintained at 37 °C in a

humidified atmosphere containing 5% CO₂. Medium was changed every 2–3 days. After approximately 2 weeks, cells were found at the edge of the tissue fragments. When colonies of fibroblast-like cells appeared and cells in wells reached 70% confluence, cultures were detached using 0.25% trypsin-EDTA, and reseeded in 10 cm dishes for optimal proliferation.

Human CB samples were obtained as described previously (4,5). Briefly, CB mononuclear cells (MNCs) were isolated using lymphocyte separation medium (1.077 g/ml) (TBD Biotech, Tianjing, China), and were immunomagnetically enriched for CD34⁺ cells using MACS CD34⁺ Cell Isolation Kit (Miltenyi Biotech Inc., Bergisch Gladbach, Germany) according to the manufacturer's instructions. Purity of CD34⁺ cells was in the order of 80–90%, determined by flow cytometry (FCM).

CD29 shRNA design, construction and packaging of shRNA vectors

The two CD29-specific small hairpin RNAs (KD1 and KD2) oligomers were designed using online RNAi design software. These shRNA sequences excluded all sequence homology with any other human coding sequences in BLAST (<http://www.ncbi.nlm.nih.gov/BLAST>). Details of shRNA sequences are provided in Table 1. Sense and antisense oligomers were used to produce double-stranded oligomers, and these were inserted into retroviral vector RNAi-pSIREN-RetroQ, which drives shRNA production from the U6 promoter and also contains puromycin resistance (Clontech, San Francisco, USA). Inserts were confirmed by sequencing (ABI PRISM 310 Genetic Analyzer, Foster, CA, USA). If not otherwise mentioned, RNAi-pSIREN-RetroQ vectors containing scrambled target sequences not complementary to any known miRNA were served as controls (CTRL). Phoenix packaging cell line was co-transfected with RNAi-pSIREN-RetroQ retroviral plasmid and viral packaging plasmid by Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA), following the manufacturer's instructions. Viral supernatants were collected at 48 or 72 h after transfection and stored at –80 °C until future use.

Table 1. Primer used in this study

Name	Sequence
KD1	CCACAGACATTACATTA
KD2	CCACAGATGCCGGTTTCAC
CTRL	ACACGTCCGAACATACTAC
CD29	F: 5'-GCATACAATTCCTTTCCTCA-3' R: 5'-AATAACCTCTACTTCTCCGT-3'

RNA extraction and real-time RT-PCR

Total RNA was extracted using trizol reagent (Invitrogen) and real-time RT-PCR analysis was performed using Bio-Rad iCycler with iQSYBER green supermix (Bio-Rad, Hercules, CA, USA) as described previously (4,5). GAPDH (glyceraldehyde-3-phosphate dehydrogenase) was the endogenous control. Standard curves for internal control and tested genes were measured each time to determine relative level of the respective transcript. Relative expression was normalized to endogenous control levels.

Co-culture of hUCMSCs with human CB CD34⁺ cells

Co-culture assay was performed as described previously (4,5). Briefly, indicated hUCMSCs were irradiated at 40 Gy, then seeded in 24-well plates (5.0×10^5 /well) overnight. CD34⁺ cells (2.0×10^4 /well) in Iscove's modified Dulbecco's medium (IMDM) (Bio-Whittaker, Walkersville, MD, USA) were supplemented with 10% FBS, 10^{-4} M 2-mercaptoethanol, 2 mM L-glutamine, 5 mg/ml insulin, 100 U/ml penicillin and 100 mg/ml streptomycin, as well as a cytokine cocktail consisting of Flt ligand (FL; 10 ng/ml), SCF (10 ng/ml), thrombopoietin (TPO, 10 ng/ml) and interleukin (IL)-6 (10 ng/ml), all of which were purchased from Peprotech Inc, Rocky Hill, NJ, USA. After 14 days culture, non-adherent and adherent haematopoietic cells loosely attached to stromal cells, were harvested by gentle pipetting, and counted.

Long-term culture-initiating cell (LTC-IC) assay

LTC-IC assay was performed as described previously (4,5). Briefly, 1.0×10^4 CB CD34⁺ cells were pipetted into six-well plates containing nearly confluent, irradiated CD29-deficient hUCMSC or control hUCMSC monolayers in LTC medium (Myelo-Cult, StemCell Inc., Vancouver, BC, Canada), along with 10^{-6} M hydrocortisone sodium hemisuccinate (Sigma, St. Louis, MO, USA). LTC medium contained horse serum, foetal bovine serum, 2-mercaptoethanol and α -MEM. 50% of the medium was replaced weekly with fresh. Both non-adherent and adherent cells were harvested weekly over 3–7 weeks culture, in complete methylcellulose medium containing 2.8% BSA, 30% FBS (Hyclone), 50 ng/ml SCF, 20 ng/ml IL-3, 20 ng/ml GM-CSF, 20 ng/ml IL-6 and 3 U/ml EPO (Kirin, Tokyo, Japan), at 37 °C with 5% CO₂. After 14–16 days, colonies with greater than 50 cells were counted, to assess LTC-IC activity. Colony-forming units (CFU-Cs), colony-forming unit-mix (CFU-Mix), colony-forming unit-erythrocyte (CFU-Es) and burst-forming unit-erythrocyte (BFU-Es) were

counted to calculate frequency of LTC-IC, according to the manufacturer's instructions (StemCell Inc.).

NOD/SCID repopulating cell (SRC) assay

Total of 5.0×10^4 CB CD34⁺ cells were co-cultured with indicated stromal cells for 4 weeks, harvested as described above, and injected intravenously (i.v.) into 8-week old, sublethally irradiated (3.5 Gy) NOD/SCID mice. Peripheral blood was obtained by non-lethal eye-bleeds under anaesthesia with isoflurane, per institutional guidelines. Mice were sacrificed 12 weeks post-transplantation. Mononuclear cells were harvested and analysed by FCM. Human cell repopulation was assessed using anti-human-CD45-fluorescein isothiocyanate (FITC; 555482), anti-human-CD34-phycoerythrin (PE; 550761), anti-human-CD36-PE-cyanin 7 (PC7; 563666) and anti-glycophorin A (GPA)-allophycocyanin (APC; 551336) antibodies. Isotype controls were mouse IgG1 conjugated to PE, APC, PC7 or FITC. All antibodies were from Becton Dickinson, Franklin L. New Jersey, USA.

Statistical analysis

Results are expressed as mean $\pm$ SD. Statistical comparisons were performed using two-tailed Student's *t*-test.

Results

Ex vivo expansion of CB CD34⁺ cells with hUCMSCs

To investigate how hUCMSCs affected proliferation and differentiation of HSCs, CB CD34⁺ cells were cultured with or without hUCMSCs for 14 days *in vitro*. As expected, total nucleate cells expanded significantly more when in cultures supported by hUCMSCs (39.40 ± 3.84 -fold versus 16.83 ± 3.84 -fold; $P < 0.05$; Fig. 1a). Then, we examined CD29 expression of hUCMSCs by FCM. Consistent with prior work, hUCMSCs constitutively highly expressed CD29 (Fig. 1b).

CD29 was required for haematopoiesis-supporting activity of hUCMSCs

As hUCMSCs expressed high levels of CD29, we first investigated whether the support of haematopoiesis by hUCMSCs resulted from CD29-mediated interaction between hUCMSCs and CB CD34⁺ cells. Interestingly, addition of soluble anti-CD29 antibody to co-cultures significantly blocked expansion of CB CD34⁺ cells, specially CD34⁺ CD38⁺ populations (Fig. 2a). Unexpectedly, although the mechanism by which

Figure 1. *Ex vivo* expansion of CB CD34⁺ cells with human umbilical cord mesenchymal stem cells (hUCMSCs). (a) Total nucleate cells expansion after 14 days co-culture with or without hUCMSCs, in the presence of cytokine cocktail. Data expressed as mean $\pm$ SE of mean ($n = 5$); * $P < 0.05$. (b) Flow cytometric analysis of CD29 expression in hUCMSCs. Blue histogram: isotype control; red histogram: specific antibody. Data shown are from one representative experiment of three reproducible experiments.

Figure 2. CD29 was required for HSC-supporting activity of human umbilical cord mesenchymal stem cells (hUCMSCs). (a) A total of 2.0×10^4 CB CD34⁺ cells were co-cultured with hUCMSCs in the presence of soluble mAb to CD29 or isotype mAb for 7 days, and harvested for flow cytometric analysis. Values indicate fold increase compared to initial number of cells. Data given as mean $\pm$ SD of three separate experiments. * $P < 0.05$, compared to cultures without antibody, or with isotype mAb. (b) Real-time RT-PCR was performed to evaluate expression level of CD29 in hUCMSCs after transduction with vectors for CD29 knockdown (KD1 or KD2), or control (CTRL). Data presented as ratio of CD29 levels in hUCMSCs, KD1 or KD2 to that in CTRL. Each reaction was performed in triplicate.

soluble isotype IgG1 promotes CD34⁺ cell expansion is not yet clear, addition of soluble isotype IgG1 led to increased expansion of CD34⁺ CD38⁺ cells. These results suggest that antibody treatment altered the culture milieu, which specifically promoted or inhibited CD34⁺ cell expansion, depending on effects on interactions between hUCMSCs and CB CD34⁺ cells. Taken together, the results strongly suggest that CD29 is important for the ability of hUCMSCs to support haematopoiesis.

To further investigate the function of CD29 on haematopoiesis support by hUCMSCs, CD29 knockdown models were created using hUCMSCs, by using retroviral vectors. CD29 levels in hUCMSCs transduced with the indicated virus were determined using real-time RT-PCR. We observed clear down-modulation of CD29 in KD cells compared to CTRL (Fig. 2b). Based on shRNA influence on CD29 expression, KD1 was chosen for further studies.

Effect of CD29 knockdown in hUCMSCs on LTC-IC activity of CB CD34⁺ cells

To test capability of CD29 of hUCMSCs in supporting self-renewal and maintaining multipotent differentiation of HSC, we performed LTC-IC assay. CB CD34⁺ cells were co-cultured with KD1 or CTRL cells in LTC-IC medium for 3–7 weeks, then subjected to CFU assay. After 14–16 days culture, colonies with more than 50 cells were counted. As shown in Fig. 3, numbers of total CFCs and CFU-GMs from cells co-cultured with KD1 cells for 3–5 weeks were similar to those from cells co-cultured with CTRL cells. However, numbers of total CFCs and CFU-GMs from cells co-cultured with KD1 cells decreased rapidly at week 6 then progressively lost their multipotency of differentiation over time of culture. CFCs and CFU-GMs were close to undetectable at week 7 co-culture. Consistently, although numbers of CFU-Mix and BFU-Es from cells co-cultured

Figure 3. Effect of CD29 knockdown in human umbilical cord mesenchymal stem cells (hUCMSCs) on long-term culture-initiating cell activity of CB CD34⁺ cells. Total of 1.0×10^4 CB CD34⁺ cells were co-cultured with KD1 or CTRL cells for 3–7 weeks, then subject to CFU assay. After 14–16 days culture, colonies, including BFU-Es, CFU-GMs, and CFU-Mix, with greater than 50 cells were counted. Results expressed as mean $\pm$ SD ($n = 5$). * $P < 0.05$, compared between KD1 and CTRL cells (Student's *t*-test).

with KD1 cells for 3–4 weeks were similar with those from cells co-cultured with CTRL cells, numbers of CFU-Mix and BFU-Es from cells co-cultured with KD1 cells decreased rapidly at week 5. It was noted that compared to CB CD34⁺ cells co-cultured with CTRL cells (which retained the capacity of multipotent differentiation for at least 6 weeks), CB CD34⁺ cells co-cultured with KD1 only retained the capacity of multipotent differentiation for 5 weeks at the most. This was because numbers of CFU-Mix and BFU-Es from these cells were not detectable at week 6. These results suggest that CD29 in hUCMSCs play an important role in the ability of hUCMSCs to support haematopoiesis.

Effect of CD29 knockdown of hUCMSCs on expansion of SRCs

To further support our *in vitro* expansion and LTC-IC results, we examined engraftment of CB CD34⁺ cells after co-cultured with KD1 or CTRL cells, in NOD/SCID mice, by temporal monitoring of peripheral blood and BM of recipients, for 12 weeks. Sublethally irradiated NOD/SCID mice were transplanted with 5.0×10^4 CB CD34⁺ cells from the co-cultures, with irradiated KD1 or CTRL cells, for 4 weeks. Although levels of

chimaerism gradually increased in the peripheral blood of mice transplanted with CB CD34⁺ cells co-cultured with CTRL cells, engraftment of CB CD34⁺ cells co-cultured with KD1 cells peaked between 2 and 4 weeks then declined (Fig. 4a). Total human cell engraftment was composed of CD45⁺, CD45[−] CD36⁺ and CD36[−] GPA⁺ cells, and CD45⁺ CD34⁺ populations were assessed in BM mononuclear cells of engrafted mice 12 weeks post-transplant, by FCM. At week 12, total human cells from mice injected with CD34⁺ cells co-cultured with KD1 was significantly lower than that of mice injected with CD34⁺ cells co-cultured with CTRL (Fig. 4b; $P = 0.026$). Statistical analysis was performed for comparison between mice injected with CD34⁺ cells co-cultured with KD1 and those injected with CD34⁺ cells co-cultured with CTRL.

We further analysed multilineage development from input CD34⁺ populations. Flow cytometric analysis of human grafts in a representative graft mouse from each group is shown in Fig. 4d. We observed significantly lower percentage of CD45⁺ cells in KD1 group compared to percentage in the CTRL group. Similarly, compared to CTRL group, KD1 group also had significantly lower percentages of CD45⁺ CD34⁺ cells (Fig. 4c). Observed multilineage development from input CD34⁺

Figure 4. Effect of CD29 knockdown in human umbilical cord mesenchymal stem cells (hUCMSCs) on repopulation of CB CD34⁺ cells in NOD/SCID mice. (a) A total of 5.0×10^4 CB CD34⁺ cells were co-cultured with KD1 or CTRL for 4 weeks and then injected intravenously into sublethally irradiated NOD/SCID mice ($n = 6$ per group). Kinetic analysis of peripheral blood engraftment (Student's *t*-test). (b) Level of total human cell engraftment. The mice were sacrificed 12 weeks after transplantation and mononuclear cells from bone marrow were analysed by flow cytometry. $P = 0.026$ compared between NOD/SCID mice injected with CD34⁺ cells co-cultured with KD1 and those injected with CD34⁺ cells co-cultured with CTRL. (c) Fraction of CD45⁺, erythroid and CD45⁺CD34⁺ population among the engrafted human cells. $*P < 0.05$. (d) Flow cytometric analysis of human CB CD34⁺ cell repopulation in a representative primary NOD/SCID mouse after co-culture with KD1 or CTRL cells. Mononuclear cells from bone marrow of engrafted NOD/SCID mice were examined for assessment of human CD45⁺ cells (R1) and erythroid cells including CD45⁻CD36⁺ (R2) and CD36⁻GPA⁺ (R3) population and CD45⁺CD34⁺ cells (R4).

populations co-cultured with KD1 cells coincided with expansion and LTC-IC assays *in vitro*. However, percentages of erythroid cells, including CD45⁻CD36⁺ and CD36⁻GPA⁺ populations in the KD1 group whose transplants of CD34⁺ cells had been co-cultured with KD1 cells had a tendency, although this was not significant, to be lower than that of mice receiving transplants of CD34⁺ cells co-cultured with CTRL cells. These results confirmed our *in vitro* data and suggest that CD29 in hUCMSCs played an important role in the ability of hUCMSCs to maintain multipotency of CB CD34⁺ cells *in vitro*.

Discussion

Self-renewal and differentiation of HSCs rely on the specified stem cell-niche interaction. Previous studies have demonstrated that human MSCs from human

umbilical cord (hUC) are able to support haematopoiesis (17,18). Some results suggest that MSCs can improve therapeutic effects of HSC transplantation in clinical treatment and haematopoietic engraftment in NOD/SCID mice (22,23). However, mechanisms involved in controlling the fate of HSCs by hUCMSCs remained largely unknown.

In this study, first we identified that hUCMSCs supported CB CD34⁺ cell expansion *in vitro* and highly expressed CD29; this is consistent with previous work (10,11,18,20,21). CD29 is an adhesion molecule; it has been used to isolate murine HSCs and other somatic stem cells (21,22), and is known to be important for HSC-niche functions. Interestingly, addition of soluble anti-CD29 antibody to co-cultures significantly blocked expansion of CB CD34⁺ cells caused by hUCMSCs, specially CD34⁺CD38⁺ populations (Fig. 2a); this is similar to our previous data resulting from FBMOb-

hTERT cells (5). This suggested that, in addition to being important for mediating HSC-niche interactions (21,24), CD29 was possibly also involved in the ability of hUCMSCs to support HSC expansion.

As expected, knockdown of hUCMSC CD29 reduced their ability to support CB CD34⁺ cell expansion long-term. CB CD34⁺ cells co-cultured with control hUCMSCs retained their capacity of multipotent differentiation for at least 6 weeks, but CB CD34⁺ cells co-cultured with CD29-deficient hUCMSCs retained theirs for only 5 weeks at the most. As CD29 is known to be important for adhesion between stem and the stromal cells of niche, CD29-deficient hUCMSCs may not interact with CB CD34⁺ cells as strongly as control hUCMSCs; this would promote efficient transition from long-term to short-term HSCs and then speed up efficient and continuous differentiation of CB CD34⁺ cells co-cultured with CD29-deficient hUCMSCs. These results also confirmed the concept that adhesion molecules expressed by haematopoietic stromal cells play key roles in their interaction with HSC (25).

Results from the LTC-IC assay were confirmed further by the engraftment experiments *in vivo*. Significantly lower percentage of CD45⁺ or CD45⁺ CD34⁺ population was observed in the CD29-deficient hUCMSCs group compared to the control group. However, percentage of erythroid cells, including CD45⁺ CD36⁺ and CD36⁺ GPA⁺ populations in the CD29-deficient hUCMSCs group (whose transplants of CD34⁺ cells had been co-cultured with CD29-deficient hUCMSCs) showed a tendency, although this was not significant, to be lower than that in mice receiving transplants of CD34⁺ cells co-cultured with control hUCMSCs. It is of note that although levels of chimaerism gradually increased in peripheral blood of mice transplanted with CB CD34⁺ cells co-cultured with control hUCMSCs, engraftment of CB CD34⁺ cells co-cultured with CD29-deficient hUCMSCs peaked between 2 and 4 weeks, then declined. These data indicate that CB CD34⁺ cells co-cultured with CD29-deficient hUCMSCs had higher engraftment and differentiation potential than CB CD34⁺ cells co-cultured with control hUCMSCs, immediately after transplant. Functional impairment of CB CD34⁺ cells co-cultured with CD29-deficient hUCMSCs *in vivo* is likely to be due to inefficient engraftment, at least in part. Successful engraftment of human HSCs *in vivo* requires suitable attachment between HSCs and the BM niche, as well as their bony migration. Mechanisms of engraftment have revealed a host of contributing factors, including adhesion molecules, such as integrins and CXCR4, an important homing factor for HSCs (26–28). Compared to CB CD34⁺ cells co-cultured with CTRL hUCMSCs, it is possible that

CB CD34⁺ cells co-cultured with CD29-deficient hUCMSCs expressed lower levels of VCAM; this leads to looser interaction between engrafted human cells and the niche. Results from BM also rule out the idea that CB CD34⁺ cells co-cultured with CD29-deficient hUCMSCs had impaired capacity to home and proliferate in the marrow, consistent with the data from engraftment of CD49f⁺HSC (25). These all demonstrate that CB CD34⁺ cells co-cultured with CD29-deficient hUCMSCs gave rise to all major haematopoietic lineages, but failed to engraft long term, indicating that after CD29 knockdown, ability of hUCMSCs to long term support HSC expansion is reduced. On the basis of our results and previous reports, we offer the concept that adhesive molecular constructs of hUCMSCs play an important role during haematopoietic development.

In summary, our data suggest the potential contribution of CD29 in hUCMSCs in expansion of haematopoiesis. Our study demonstrated that, in addition to being important for mediating HSC-niche interactions, CD29 is also possibly involved in the ability of hUCMSCs to support HSC expansion.

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References

- 1 Amsellem S, Pflumio F, Bardinet D, Izac B, Charneau P, Romeo P, *et al.* (2003) Ex vivo expansion of human hematopoietic stem cells by direct delivery of the HOXB4 homeoprotein. *Nat. Med.* **9**, 1423–1427.
- 2 Krosl J, Austin P, Beslu N, Kroon E, Humphries RK, Sauvageau G (2003) In vitro expansion of hematopoietic stem cells by recombinant TAT-HOXB4 protein. *Nat. Med.* **9**, 1428–1432.
- 3 Liu Y, Yi L, Zhang X, Gao L, Zhang C, Feng YM, *et al.* (2011) Cotransplantation of human umbilical cord blood-derived stromal cells enhances hematopoietic reconstitution and engraftment in irradiated BABL/c mice. *Cancer Biol. Ther.* **11**, 84–94.
- 4 Yang Y, Ma W, Wu D, Huang Y, Li H, Zou J, *et al.* (2013) MiR-17 partly promotes hematopoietic cell expansion through augmenting HIF-1 α in osteoblasts. *PLoS One* **8**, e70232.
- 5 Yang YX, Miao ZC, Zhang HJ, Wang Y, Gao JX, Feng MF (2007) Establishment and characterization of a human telomerase catalytic subunit-transduced fetal bone marrow-derived osteoblastic cell line. *Differentiation* **75**, 24–34.
- 6 Fong CY, Gauthaman K, Cheyyatraivendran S, Lin HD, Biswas A, Bongso A (2012) Human umbilical cord Wharton's jelly stem cells and its conditioned medium support hematopoietic stem cell expansion ex vivo. *J. Cell. Biochem.* **13**, 658–668.
- 7 Ito Y, Hasaoda H, Kitajima T, Kiyono T (2006) Ex vivo expansion of human cord blood hematopoietic progenitor cells using glutaraldehyde-fixed human bone marrow stromal cells. *J. Biosci. Bioeng.* **5**, 467–469.

- 8 Li N, Feugier P, Serrurier B, Latger-Cannard V, Lesesve JF, Stoltz JF, *et al.* (2007) Human mesenchymal stem cells improve ex vivo expansion of adult human CD34⁺ peripheral blood progenitor cells and decrease their allostimulatory capacity. *Exp. Hematol.* **3**, 507–515.
- 9 Almeida-Porada G, Porada CD, Tran N, Zanjani ED (2003) Co-transplantation of human stromal cell progenitors into preimmune fetal sheep results in early appearance of human donor cells in circulation and boosts cell levels in bonemarrow at later time points after transplantation. *Blood* **11**, 3620–3627.
- 10 Friedman R, Betancur M, Boissel L, Tuncer H, Cetrulo C, Klingemann H (2007) Umbilical cord mesenchymal stem cells: adjuvants for human cell transplantation. *Biol. Blood Marrow Transplant.* **12**, 1477–1486.
- 11 Bakhshi T, Zabriskie RC, Bodies K, Ramin S, Laura A, Paganessi LA (2008) Mesenchymal stem cells from the Wharton's jelly of umbilical cord segments provide stromal support for the maintenance of cord blood hematopoietic stem cells during long-term ex vivo culture. *Transfusion* **48**, 2638–2644.
- 12 Friedenstein AJ, Piatetzky-Shapiro II, Petrakova KV (1966) Osteogenesis in transplants of bone marrow cells. *J. Embryol. Exp. Morphol.* **3**, 381–390.
- 13 Minguell JJ, Erices A, Conget P (2001) Mesenchymal stem cells. *Exp. Biol. Med.* **6**, 507–520.
- 14 Lazennec G, Jorgensen C (2008) Concise review: adult multipotent stromal cells and cancer: risk or benefit? *Stem Cells* **6**, 1387–1394.
- 15 Troyer DL, Weiss ML (2008) Concise review: Wharton's Jelly-derived cells are a primitive stromal cell population. *Stem Cells* **3**, 591–599.
- 16 Secco M, Zucconi EN, Vieira NM, Fogaca LL, Cerqueira A, Carvalho MD, *et al.* (2008) Multipotent stem cells from umbilical cord: cord is richer than blood!. *Stem Cells* **1**, 146–150.
- 17 Chao YH, Tsai C, Peng CT, Wu HP, Chan CK, Weng T, *et al.* (2011) Cotransplantation of umbilical cord MSCs to enhance engraftment of hematopoietic stem cells in patients with severe aplastic anemia. *Bone Marrow Transplant.* **10**, 1391–1392.
- 18 Alakel N, Jing D, Muller K, Bornhauser M, Ehninger G, Orde-mann R (2009) Direct contact with mesenchymal stromal cells affects migratory behavior and gene expression profile of CD133⁺ hematopoietic stem cells during ex vivo expansion. *Exp. Hematol.* **37**, 504–513.
- 19 Campagnoli C, Roberts IA, Kumar S, Bennett PR, Bellantuono I, Fisk NM (2001) Identification of mesenchymal stem/progenitor cells in human first-trimester fetal blood, liver, and bone marrow. *Blood* **15**, 2396–2402.
- 20 Haug JS, He XC, Grindley JC, Wunderlich JP, Gaudenz K, Ross JT, *et al.* (2008) N-cadherin expression level distinguishes reserved versus primed states of hematopoietic stem cells. *Cell Stem Cell* **2**, 367–379.
- 21 Benveniste P, Frelin C, Janmohamed S, Barbara M, Herrington R, Hyam D, *et al.* (2010) Intermediate-term hematopoietic stem cells with extended but time-limited reconstitution potential. *Cell Stem Cell* **6**, 48–58.
- 22 Liu RH, Li YQ, Zhou WJ, Shi YJ, Ni L, Liu GX (2014) Supplementing mesenchymal stem cells improves the therapeutic effect of hematopoietic stem cell transplantation in the treatment of murine systemic lupus erythematosus. *Transplant. Proc.* **46**, 1621–1627.
- 23 Milazzo L, Vulcano F, Barca A, Macioce G, Paldino E, Rossi S, *et al.* (2014) Cord blood CD34⁺ cells expanded on Wharton's jelly multipotent mesenchymal stromal cells improve the hematopoietic engraftment in NOD/SCID mice. *Eur. J. Haematol.* doi: 10.1111/ejh.12363.
- 24 Stingl J, Eirew P, Ricketson I, Shackleton M, Vaillant F, Choi D, *et al.* (2006) Purification and unique properties of mammary epithelial stem cells. *Nature* **439**, 993–997.
- 25 Notta F, Doulatov S, Laurenti E, Poepl A, Jurisica I, Dick JE (2011) Isolation of single human hematopoietic stem cells capable of long-term multilineage engraftment. *Science* **8**, 218–221.
- 26 Gold J, Valinski HM, Hanks AN, Ballen KK, Hsieh CC, Becker PS (2006) Adhesion receptor expression by CD34⁺ cells from peripheral blood or bone marrow grafts: correlation with time to engraftment. *Exp. Hematol.* **34**, 680–687.
- 27 Lapidot T, Petit I (2002) Current understanding of stem cell mobilization: the roles of chemokines, proteolytic enzymes, adhesion molecules, cytokines, and stromal cells. *Exp. Hematol.* **30**, 973–981.
- 28 Teixidó J, Hemler ME, Greenberger JS, Anklesaria P (1992) Role of beta 1 and beta 2 integrins in the adhesion of human CD34hi stem cells to bone marrow stroma. *J. Clin. Invest.* **90**, 358–367.