ELSEVIER

Blood 142 (2023) 2680–2681

The 65th ASH Annual Meeting Abstracts

POSTER ABSTRACTS

501.HEMATOPOIETIC STEM AND PROGENITOR CELLS AND HEMATOPOIESIS: BASIC AND TRANSLATIONAL

iPSC-Derived Mesenchymal Stem Cells Supports the *Ex Vivo* expansion of Human Hematopoietic Stem and Progenitor Cells Via Sterile Inflammation Pathway

Maria Alejandra Kanashiro[1], *Sudip Kumar Paul, MSc*[1], *Masamitsu Sone, PhD*[1], *Ikuyo Yoshino*[1], *Kyoko Tsujimura*[1], *Makoto Ikeya, PhD*[2], *Sou Nakamura, PhD*[2], *Koji Eto, MDPhD*[1,2], *Naoya Takayama, MDPhD*[1]

[1] Department of Regenerative Medicine, Chiba University, Chiba, Japan
[2] Centre of iPS Cell Research and Application, Kyoto University, Kyoto, Japan

Ex-vivo expansion of human hematopoietic stem/progenitor cells (HSPCs) represents a promising technology for investigating HSPCs' function and overcoming donor shortage for transplantation. HSPCs appropriately regulate self-renewal and differentiation, relying on hematopoietic supporting cells within the bone marrow microenvironment. Notably, various strategies employing primary mesenchymal stem cells (MSCs) as hematopoietic supporting cells have demonstrated successful HSPC expansion (De Lima, et al, N. Engl. J. Med. 2012). However, the preparation of primary MSCs includes harmful procedures, hindering the stable supply of high-quality hematopoietic supporting cells, thereby limited expansion capability and enhancement of clone variation.

To overcome these issues, we have established the high-quality type of an immortalized MSCs (imMSCs) cell line derived from human induced pluripotent cells (iPSCs) with knockdown of p53 gene. The imMSCs exhibited exponential proliferation over 2 months, while retaining their MSC phenotype and functionality. To examine the supporting capability of imMSCs on HSPC expansion, we performed coculture experiments with cord blood-derived CD34 $^{+}$ cells in the presence or absence of combination of small compounds StemRegenin-1 and UM171 (SU). We compared four distinct culture conditions, *i.e.*, i) the basic culture with cytokines (SCF, THPO, and FLT3L), as a control; ii) cocultured with imMSCs (imMSC condition); iii) SU alone (SU condition); and iv) cocultured with imMSC and SU (imMSC+SU condition). After a 7-day culture period, we observed a 2 to 3-fold expansion of the CD34 $^{+}$CD45RA $^{-}$CD90 $^{+}$ (phenotypic HSC) population in the imMSC condition compared to control. Moreover, the addition of SU (imMSC+SU condition) demonstrated a synergistical effect in promoting HSC expansion (up to 9-fold).

Meanwhile, to evaluate the functional potential of expanded HSPCs *in vivo*, we conducted a xenotransplantation assay using NOG mice as recipients. The expanded HSPCs obtained from the initial 1,000 CD34 $^{+}$ cells were transplanted into the femoral bone of sub-lethally irradiated mice (2Gy). After 12 weeks, mice were sacrificed, and the proportion of human CD45 $^{+}$ cells in the murine bone marrow were analyzed with flow cytometer. All experimental conditions (ii, iii, and iv in the above) enabled to increase chimerism levels compared to the control (i). Notably, the imMSC+SU condition showed a significantly higher chimerism level compared to that of the freshly isolated CD34 $^{+}$ cells, highlighting the efficacy of our expanded HSPCs. Furthermore, a limiting dilution assay using immunodeficient mice revealed that the imMSC, SU, and imMSC+SU conditions showed 3.5-fold, 7.4-fold, and 20-fold expansion of functional HSCs, respectively, in comparison to the control.

To elucidate the underlying mechanism how intervention promote HSPC expansion, we employed RNA sequencing analysis. We found that interferon signaling genes (ISGs) were significantly elevated in HSPCs from either imMSC or imMSC+SU compared to other conditions. This finding of elevated ISGs was recapitulated by freshly isolated CB-derived HSCs (uncultured), indicating that ISGs may contribute to the self-renewal ability of both uncultured HSC and those cocultured with imMSCs.

While multiple roles of inflammatory signaling, including ISGs, have been identified in hematopoiesis, its role in the fate decision of cord blood-derived HSCs remains poorly understood. To gain further insights, we examined the role of the IFN pathway through shRNA-mediated knockdown of IRF3/7 in CD34 $^{+}$ HSPCs. Our results revealed that the knockdown of IRF3/7 in HSPCs led to a significant reduction in the phenotypic HSC population in all conditions, suggesting the critical role of interferon signaling in HSC expansion. In this context, we attempted to recapitulate the effects of imMSCs by administering recombinant IFN-2α with various doses. However, IFN-2α administration at any doses failed to promote HSC expansion, suggesting transcript of ISGs-dependent but not IFN protein-dependent mechanism might be crucial for the HSC self-renewal. Taken together, our new co-culture system using imMSCs should contribute to a better understanding of the regulatory networks governing HSPC biology as well as the future clinical application of HSPC expansion.

Disclosures Eto: *Otsuka Pharmaceuticals Co., Ltd.:* Research Funding; *Megakaryon Inc.:* Research Funding.

https://doi.org/10.1182/blood-2023-181378