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Fucosylation with fucosyltransferase VI or fucosyltransferase VII improves cord blood engraftment

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Abstract

Background aims. Advantages associated with the use of cord blood (CB) transplantation include the availability of cryopreserved units, ethnic diversity and lower incidence of graft-versus-host disease compared with bone marrow or mobilized peripheral blood. However, poor engraftment remains a major obstacle. We and others have found that *ex vivo* fucosylation can enhance engraftment in murine models, and now *ex vivo* treatment of CB with fucosyltransferase (FT) VI before transplantation is under clinical evaluation (NCT01471067). However, FTVII appears to be more relevant to hematopoietic cells and may alter acceptor substrate diversity. The present study compared the ability of FTVI and FTVII to improve the rapidity, magnitude, multi-lineage and multi-tissue engraftment of human CB hematopoietic stem and progenitor cells (HSPCs) *in vivo*. *Methods.* CD34-selected CB HSPCs were treated with recombinant FTVI, FTVII or mock control and then injected into immunodeficient mice and monitored for multi-lineage and multi-tissue engraftment. *Results.* Both FTVI and FTVII fucosylated CB $CD34^+$ cells *in vitro*, and both led to enhanced rates and magnitudes of engraftment compared with untreated CB $CD34^+$ cells *in vivo*. Engraftment after treatment with either FT was robust at multiple time points and in multiple tissues with similar multi-lineage potential. In contrast, only FTVII was able to fucosylate T and B lymphocytes. *Conclusions.* Although FTVI and FTVII were found to be similarly able to fucosylate and enhance the engraftment of CB $CD34^+$ cells, differences in their ability to fucosylate lymphocytes may modulate graft-versus-tumor or graft-versus-host effects and may allow further optimization of CB transplantation.

Key Words: *cord blood hematopoietic stem and progenitor cells, FTVI, FTVII, fucosylation, improved engraftment*

Introduction

For effective homing and engraftment of hematopoietic stem and progenitor cells (HPSCs) to the bone marrow (BM), it is thought that specific cell surface ligands expressed by the HSPCs interact with receptors expressed by the endothelial cells lining the blood vessels of the hematopoietic system. Although the expression of certain cell surface glycoproteins by HSPCs might be sufficient for homing to hematopoietic tissues (1–9), there is evidence that the activity of specific ligands is improved when they are fucosylated (10–17). Fucosylation is the addition of fucose moieties by fucosyltransferase (FT)-directed, site-specific processes. Previous studies revealed that CB HSPCs have consistently lower levels of endogenous fucosylation than BM or mobilized peripheral blood progenitor cells and that *ex vivo* fucosylation using recombinant human FTVI enhances the adhesion, homing and engraftment of CB HSPCs in xenografts (14,18). These data suggest that *ex vivo* fucosylation using FTVI may be used to mitigate the delayed engraftment that is currently associated with

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CB transplantation. A clinical trial is underway testing the effect of *ex vivo* fucosylation of CB using recombinant human FTVI before transplantation (NCT01471067).

FTVI is not normally expressed on hematopoietic cells, but rather in endothelial, epithelial, gastrointestinal and some malignant cells. In contrast, FTVII is widely expressed on hematopoietic cells including BM $CD34^+$ cells (19). FTVII appears to be the dominant FT responsible for producing leukocyte selectin ligand activity (20), and a spontaneous FTVII mutation impairs selectin binding (21). FTVII expression is unexpectedly low in CB HSPCs (22), suggesting that fucosylation with FTVII may provide a more physiologic approach to restoring fucosylated proteins to CB HSPCs. The aim of the present study was to compare the activities of FTVI and FTVII to identify any qualitative differences in the rate, magnitude, multi-lineage and multi-tissue engraftment of human CB HSPCs *in vivo*.

Methods

Two α-(1,3)-fucosyltransferase enzymes, FTVI and FTVII (provided by American Stem Cell Inc, Floresville, TX, USA), were compared for their ability to fucosylate CB HSPCs in an *ex vivo* setting. Fucosylation was revealed by flow cytometry through the binding of HECA-452 (BD Biosciences, San Jose, CA, USA), a directly conjugated (fluorescein isothiocyanate) rat immunoglobulin M antibody that reacts against fucosylated (sialyl Lewis X-modified) cell surface glycoproteins, including P-selectin glycoprotein ligand-1 (CD162) (10). HECA-452 was originally described as detecting a cutaneous lymphocyte antigen.

Hematopoietic cells

Fresh CB units were obtained and all animal work was conducted under protocols approved by M.D. Anderson Cancer Center institutional review board and institutional animal care and use committee. CB mononuclear cells (MNCs) were isolated from fresh CB units by Ficoll-Histopaque density separation and CB $CD34^+$ cells enriched by magnetic-activated cell sorting according to manufacturer's instructions (Miltenyi Biotec, Auburn, CA, USA). Magnetic-activated cell sorting-selected $CD34^+$ cells were pooled and divided into (i) untreated, (ii) FTVI-treated or (iii) FTVII-treated fractions. *Ex vivo* fucosylation was performed as previously described (18). Briefly, CB $CD34^+$ cells were treated at 10^6 cells/mL for 30 min at room temperature with 1 mmol/L guanosine diphosphate β-fucose (EMD Biosciences, San Diego, CA, USA) in phosphate-buffered saline containing 1% human serum albumin (HSA; Baxter Healthcare Corporation, Westlake Village, CA, USA) and in the previously optimized concentrations of 100 mU/mL FTVI, or 75 μg/mL FTVII. Untreated cells were incubated in the same manner except that no enzyme was added. After incubation, cells were washed in phosphate-buffered saline containing 1% HSA, cellularity was determined by hemacytometer and cells were diluted in saline before intravenous injection into sub-lethally irradiated NOD-SCID IL-2Rγ^{null} (NSG) mice (Jackson Laboratories, Bar Harbor, ME, USA). A cesium-137 source delivered a total sub-lethal radiation dose of 300 cGy over 1 min (Mark I-25 Irradiator; J.L. Shepherd and Associates, San Fernando, CA, USA). In each group (n = 5 mice/group), mice received 10^5 CB $CD34^+$ cells.

Assessment of human engraftment in peripheral blood, BM and spleen of NSG recipients

Human engraftment was determined once or twice per week by withdrawal of 40 μL of peripheral blood from the retro-orbital sinus of anesthetized mice and lysis of red blood corpuscles (Pharm Lyse; BD PharMingen, San Jose, CA, USA). At >100 days after transplantation, BM and spleen were also harvested. Samples were assessed for the presence of human and murine $CD45^+$ cells by flow cytometry (FACSCalibur; BD Biosciences) using phycoerythrin-conjugated rat anti-mouse CD45 and APC-conjugated mouse anti-human CD45 (both BD Biosciences). Mouse peripheral blood, human CB and antibody isotypes provided appropriate controls. Data were acquired and analysis was performed using CellQuest Pro software (BD Biosciences). The percentage of human engraftment was calculated as follows: [Percent human CD45 ÷ (percent human CD45 + percent murine CD45)] × 100.

Secondary transplantation of human $CD34^+$ cells from BM of primary CB $CD34^+$ recipients

Recipients of FTVI-treated or FTVII-treated CB $CD34^+$ cells were euthanized at >100 days after transplantation (as previously described), and femoral BM from individuals in each group (primary recipients) was pooled and transplanted into three groups of sub-lethally irradiated NSG mice (n = 5 mice/group, secondary recipients). Equivalent numbers of nucleated BM cells (approximately 3×10^7/mouse) were transplanted intravenously, and the number of human $CD34^+$ cells (approximately 2×10^6/mouse) was retrospectively assessed by flow cytometry of the pooled BM samples (as previously described). Mice were euthanized >10 weeks after