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Short report

Novel source of human hematopoietic stem cells from peritoneal dialysis effluents

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ABSTRACT

Hematopoietic stem cells (HSCs) hold great promise for the treatment of various diseases and blood disorders. However, limited availability of these cells has hampered their applications in clinical and biological research. Here we have identified a new source of autologous human HSCs in peritoneal dialysis (PD) effluents from patients with end stage renal diseases (ESRDs). Cells isolated from PD effluents contain a $Lin^-/CD34^+/CD38^-/CD90^+$ sub-population and can repopulate NOD/SCID/gamma −/− mice in serial transplantation. Differing from cord blood HSCs, PD-derived HSCs have high tendencies to repopulate peritoneal cavity and spleen with myeloid cells and B lymphocytes. Repopulating HSCs also reside in peritoneal cavities in mice. The isolation of HSCs from peritoneal cavities provides a novel and promising source of autologous and functional HSCs for stem cell research and possible clinical use.

1. Introduction

Hematopoietic stem cells (HSCs), defined by their ability to self-renew and differentiate into all blood cell types (Till and Mc, 1961; Seita and Weissman, 2010), have been isolated from bone marrow (Mathe et al., 1963), mobilized peripheral blood (Gianni et al., 1989), and umbilical cord blood (Gluckman et al., 1989). They hold great promise for the treatment of many diseases, including selected hematologic malignancies, immunodeficiencies, hemoglobinopathies, bone marrow failure syndromes, and congenital metabolic disorders (Bryder et al., 2006). Previous studies indicate that, during development, hematopoiesis takes place in a variety of sites including yolk sac, aorta–gonad–mesonephros, liver, spleen, and bone marrow. In adulthood, HSCs mainly reside in the bone marrow, and also exist in extramedullary organs including spleen and liver, especially during immune responses following infection or during failed marrow hematopoiesis (Kim, 2010). Identification of additional sources of HSCs will further our understanding of the biology of HSCs and the interaction between stem cells and their microenvironment, and lead to the development of new strategies of HSC-based therapies.

Our recent studies have revealed that subcutaneous implantation of biomaterials promotes the recruitment of HSC to the implantation sites (Nair et al., 2011). Although the mechanism(s) of biomaterial-mediated stem cell recruitment is unclear, it is likely that inflammatory products released during foreign body reactions prompt the recruitment of HSCs. To test whether such observations also hold true in humans, we examined patients with end stage renal disease (ESRD) who would undergo peritoneal dialysis (PD). Since PD patients have a catheter implanted in the peritoneum prior to the treatment, we tested whether dialysate effluents from incident PD patients contained HSCs. By analyzing cells isolated from PD effluents following catheter implantation, we identified a $Lin^-/CD34^+/CD38^-/CD90^+$ sub-population of cells in PD effluents. The hematopoietic multipotency of PD effluent-isolated HSCs was assessed using a transplantation assay. Concordant with this result, we also identified functional HSCs from mouse peritoneal cavity.

2. Materials and methods

2.1. Study population

Peritoneal dialysis (PD) fluid samples were collected from 49 ERSD patients visiting the University of Texas Southwestern Medical Center/DaVita PD Clinic (Irving, TX) within a week after catheter implantation

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for post-operative catheter care and PD training. For PD procedure the catheter was flushed with 1–2 l of dialysate solution (2.5% Dianeal PD solution, Baxter, Deerfield, IL), and the effluent was collected for experiment at the PD clinic after each cycle. The protocol of PD effluent collection was approved by the Institutional Review Board of the University of Texas Southwestern Medical Center at Dallas. Patients with peritonitis were excluded from this study. Of all 49 patients, 12 had been on hemodialysis (HD) for different periods of time prior to commencement of PD, while 37 patients had no prior HD treatment. The demographic information of the patients is listed in Table 1.

2.2. Flow cytometry analyses of human PD effluent samples

For flow cytometry analysis, some of the recovered cells were followed by lysis of RBC using RBC lysing buffer (Sigma Chemical Co., St Louis, MO) according to the manufacturer's instructions. For RBC lysis, 5 ml of RBC Lysing Buffer was gently mixed and incubated with the cell pellet collected from a patient effluent at room temperature for 10 min. The mixture was diluted with 20 ml of medium supplemented with FBS, and the cells were collected after centrifugation at 250–500*g* for 7 min. Cells were then washed in PBS, counted and adjusted to 5×10^6/ml in ice cold PBS with 2% FBS, and stained with monoclonal antibodies anti-human APC-CD90, FITC-CD34, PE-CD38 (BD Bioscience, San Jose, CA), Biotin-Lineage cocktail (Miltenyi Biotec Inc, Auburn, CA), and PE-Cy5.5-Strepavidin secondary antibody (BD Bioscience, San Jose, CA) for HSCs staining. PE-CD3, PE-CD19, PE-CD20, FITC-CD15, FITC-CD66b, PE-CD45, and PE-CD71 (BD Bioscience, San Jose, CA) were also used for hematopoietic lineage staining. FITC-CD34, PE-CD38 (BD Bioscience, San Jose, CA), Biotin-Lineage kit (Miltenyi Biotec Inc, Auburn, CA), PE-Cy5.5-Strepavidin secondary antibody (BD Bioscience, San Jose, CA), APC-CD10, eFluo 450-CD45RA, and APC-CD123 were used for progenitor cell staining. Stained cells were analyzed on BD FACSCalibur or FACSAria (BD Bioscience, San Jose, USA) for the presence of various hematopoietic populations.

Table 1
Demographic characteristics of peritoneal dialysis patients.

Patient characteristics	Number
Male	28
Females	21
Total	49
Median age (years)	52
Dialysis history	
Prevalent patients	0
Race	
African American	24
Caucasian	10
Hispanics	12
Asian/Pacific Islander	3
Prior hemodialysis among incident patients	
Prior hemodialysis	12
No prior hemodialysis	37
Causes of end stage kidney disease	
Diabetes mellitus	22
Hypertension	12
Primary glomerulonephritis	2
IgA nephropathy (1)	
Chronic glomerulonephritis (1)	
Lupus nephritis	2
Autosomal dominant PKD	4
HIV nephropathy	2
Failed Kidney transplant	2
Chronic interstitial nephritis	0
Hepatitis C associated nephropathy	2
Unknown cause	1

2.3. In vivo repopulation in NSG mice

For the repopulation study, non-obese diabetic (NOD)-*scid IL2rγ*^{null} (NSG) mice were purchased and maintained at the University of Texas Southwestern Medical Center animal facility. All animal experiments were performed with the approval of University of Texas Southwestern Committee on Animal Care.

To study the hematopoietic multipotency of the cells derived from PD effluents, freshly recovered peritoneal cells ($1–10 \times 10^7$ cells per animal) were injected intraperitoneally or intravenously via the retro-orbital route into sub-lethally irradiated (2.5 Gy) 8- to 10-week-old NSG mice. Eight weeks after transplantation, bone marrow cells from the recipient mice were analyzed by flow cytometry for the presence of human cells. Monoclonal anti-human PE-CD45, PE-CD71, FITC-CD15, FITC-CD66b, PE-CD19, PE-CD20, Biotin-CD3, APC-streptavidin secondary antibody, and FITC-CD34 antibodies (BD Bioscience, San Jose, CA) were used for the staining of human myeloid, lymphoid, and primitive cells (Zhang et al., 2008). To compare different repopulation rates in organs, peritoneal lavage cells and spleen cells were also isolated from a few NSG recipient mice for lineage staining.

To evaluate long term reconstituting potential of PD derived HSCs, bone marrow aspirates from one hind leg of a primary recipient, or peritoneal lavage cells, or spleen cells from a primary recipient were transplanted into a secondary recipient (Zhang et al., 2008; Hogan et al., 2002). For limiting-dilution analysis, mice were considered positive for human HSC engraftment when at least 1% (for primary transplantation) or 0.1% (for secondary transplantation) CD45/71 human cells were detected among the mouse bone marrow cells (Zhang et al., 2008).

2.4. Hematopoietic colony assays

Freshly isolated peritoneal cells followed by RBC lysis were washed in PBS and diluted to 1×10^6/ml in Iscove's modified Dulbecco's medium (IMDM) with 2% FBS, and then seeded into methylcellulose medium H4436 (StemCell Technologies) for CFU-GEMM, CFU-GM, and BFU-E colony formation, according to the manufacturer's protocols (Zheng et al., 2011a; Zhang et al., 2006).

2.5. EGFP transgenic mice peritoneal cell transplantation and repopulation study

For the mice peritoneal cell transplantation study, EGFP negative C57BL/6 mice (6–8 weeks old) were used and maintained at the University of at Arlington animal facility. The animal use protocol was approved by the Institutional Animal Care and Use Committee of the University of Texas at Arlington. EGFP negative C57BL/6 mice (6–8 weeks old) were irradiated (whole body X-ray irradiation) at 1000 cGy and then transplanted with sex matched peritoneal cells isolated from EGFP transgenic mice through retro-orbital injection. Eight weeks after transplantation, peripheral blood and peritoneal cells of recipient mice were isolated for flow cytometry analysis for GFP$^+$ hematopoietic lineage markers Thy1.2, B220, Mac-1, and Ter119 essentially as we described (Kang et al., 2015). Eighteen weeks after transplantation, peripheral blood, peritoneal cells, bone marrow cells, and spleen cells of recipient mice were collected for GFP$^+$ lineage marker analysis again to check long-term peritoneal HSCs.

2.6. Statistical analysis

Data are expressed as Mean ± SEM. IBM SPSS Statistics 19 software was used for analysis. Significant levels were calculated using student's t-test. One-way ANOVA and post-hoc Scheffe's test were used for comparisons between multiple groups. Differences were considered significant when $p < 0.05$.