

JAK2V617F-bearing vascular niche enhances malignant hematopoietic regeneration following radiation injury

Chi Hua Sarah Lin,^{1*} Yu Zhang,^{2*} Kenneth Kaushansky³ and Huichun Zhan^{1,4}

¹Department of Medicine, Stony Brook School of Medicine, NY, USA; ²Biopharmaceutical R&D Center, Chinese Academy of Medical Sciences & Peking Union Medical College, Suzhou, China; ³Office of the Sr. Vice President, Health Sciences, Stony Brook School of Medicine, NY, USA and ⁴Northport VA Medical Center, Northport, NY, USA

**CHSL and YZ contributed equally to this work.*

©2018 Ferrata Storti Foundation. This is an open-access paper. doi:10.3324/haematol.2017.185736

Received: December 1, 2017.

Accepted: March 14, 2018.

Pre-published: March 22, 2018.

Correspondence: huichun.zhan@stonybrookmedicine.edu or Huichun.Zhan@va.gov

Materials and Methods

Complete blood counts and colony assays

Complete blood counts and hematopoietic colony formation assays were performed as we previously described.¹

Flow cytometry

Marrow cell flow cytometric sorting was performed on a FACSAria™ III (BD Biosciences, San Jose, CA, USA). CD45 (Clone 104) (Biolegend, San Diego, CA, USA), CD45.1 (Clone A20) (BD Biosciences), CD45.2 (Clone 104) (Biolegend), EPCR (CD201) (Clone eBio1560, eBioscience, San Diego, CA, USA), CD150 (Clone mShad150, eBioscience), and CD48 (Clone HM48-1, Biolegend) antibodies were used to enumerate CD45⁺EPCR(CD201)⁺CD150⁺CD48⁻ (E-SLAM) cells.¹

Cell apoptosis was analyzed by flow cytometry using the Annexin V Apoptosis Detection Kit I (BD Biosciences) according to manufacturer's protocol. For cell cycle analysis, cells were fixed in 70% ethanol at 4°C for at least 1 h. The cells were washed multiple times then stained with 50 µg/ml propidium iodide (BD Biosciences) for 15 min at room temperature and analyzed using a FACSCalibur flow cytometer (BD Biosciences). Data were analyzed using FlowJo software (Tree Star, Ashland, OR, USA).

For analysis of phosphorylation of the Tyr-1173 residue of epidermal growth factor receptor (EGFR), marrow CD150⁺CD48⁻ HSPCs were fixed and permeabilized using the Cytofix/Cytoperm kit following the manufacturer's instructions (BD Biosciences). Cells were stained with anti-phospho-Tyr-1173 rabbit anti-mouse polyclonal antibody (ThermoFisher Scientific, Waltham, MA USA), followed by a secondary goat anti-rabbit Alexa Fluor 488 antibody (ThermoFisher Scientific).

Isolation of murine lung endothelial cells

Primary murine lung EC isolation were performed as we previously described.¹⁻³ Briefly, mice were euthanized and the chest was immediately opened through a midline sternotomy. The left ventricle was identified and the ventricular cavity was entered through the apex with a 27-gauge needle. The right ventricle was identified and an incision was made in the free wall to exsanguinate the animal and to allow the excess perfusate to exit the vascular space. The animal was perfused with 30 ml of cold PBS. The lung tissue was collected and minced finely with scissors. The tissue fragments were digested in DMEM medium containing 1 mg/mL Collagenase D (Roche, Switzerland), 1 mg/mL Collagenase/Dispase (Roche) and 25 U/mL DNase (Sigma, St. Louis, MO) at 37°C for 2hr with shaking, after which the suspension was homogenized by passaging through a 16-gauge needle several times. The homogenate was filtered through a 70µm nylon mesh (BD Biosciences, San Jose, CA) and pelleted by centrifugation (400g for 5 min). Cells were first depleted for CD45⁺ cells (Miltenyi Biotec) and then positively selected for CD31⁺ cells (Miltenyi Biotec) using magnetically labeled microbeads according to the manufacturer's protocol. Isolated ECs (CD45⁻CD31⁺) were cultured in EC culture medium with no medium change for the first 72hrs to allow EC attachment followed by medium change every 2-3 days. Cells were re-selected for CD31⁺ cells when they reach >70-80% confluence (usually after 3-4 days of culture). Although we do not have a definitive answer as to whether these CD45⁻CD31⁺ lung ECs are true endothelial progenitors with capacity to repopulate blood vessels *in vivo*,⁴ these cells display many endothelial features including ingestion of acetylated low density lipoprotein, expression of typical endothelial antigens, and capability of *in vitro* proliferation and tube formation as we previously described.¹⁻³

In vitro cultures

ECs were cultured on 1% gelatin coated 48-well plate in complete EC medium as we previously described.¹ To test the effect of ECs on HSPC radioprotection, 1×10^4 *JAK2WT* or *JAK2V617F* Lin⁻ HSPCs were cultured on EC (*JAK2WT* or *JAK2V617F*) monolayer in StemSpan® serum-free expansion medium (SFEM) containing 100 ng/mL recombinant mouse Stem Cell Factor (SCF), 6 ng/mL recombinant mouse IL3, and 10 ng/mL recombinant human IL-6 (all from Stem Cell Technologies, Vancouver, BC) overnight and irradiated with 300cGy the next morning. 24 hours after irradiation, cells were counted and plated for hematopoietic colony formation assay.

Quantitative Real-Time Polymerase Chain Reaction

The TaqMan® Gene Expression Assay (ThermoFisher Scientific) was used for real-time quantitative polymerase chain reaction (qPCR) to verify differential expression of SCF, Chemokine (C-X-C motif) ligand 12 (CXCL12), epidermal growth factor (EGF), and pleiotrophin (PTN) on an Applied Biosystems 7300 Real Time PCR System (ThermoFisher Scientific). The gene expression levels were normalized to Actin beta (Actb) expression and relative fold changes was calculated by the $2^{-\Delta\Delta CT}$ method. All assays were performed in triplicate.

Statistical Analysis

Statistical analyses were performed using Student's unpaired, 2-tailed *t* tests using Excel software (Microsoft). Repeated ANOVA models were used to compare blood counts and chimerism between Tie4/FF1 recipients and control recipients following marrow transplantation. A *p* value of less than 0.05 was considered significant. For all bar graphs, data are presented as mean \pm standard error of the mean (SEM).

1. Zhan H, Ma Y, Lin CH, Kaushansky K. JAK2V617F-mutant megakaryocytes contribute to hematopoietic stem/progenitor cell expansion in a model of murine myeloproliferation. *Leukemia* 2016.
2. Lin CH, Kaushansky K, Zhan H. JAK2V617F-mutant vascular niche contributes to JAK2V617F clonal expansion in myeloproliferative neoplasms. *Blood Cells Mol Dis* 2016;62:42-8.
3. Zhan H, Lin CHS, Segal Y, Kaushansky K. The JAK2V617F-bearing vascular niche promotes clonal expansion in myeloproliferative neoplasms. *Leukemia* 2017.
4. Basile DP, Yoder MC. Circulating and tissue resident endothelial progenitor cells. *J Cell Physiol* 2014;229:10-6.